THE PRINCIPLES O.
INSECT PHYSIOLO

by

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PREFACE

INSECTS PROVIDE an ideal medium in which to study all the problems of physiology. But if this medium is to be used to the best advantage, the principles and peculiarities of the insect's organization must be first appreciated. It is the purpose of this book to set forth these principles so far as they are understood at the present day. There exist already many excellent text-books of general entomology; notably those of Imms, Weber and Snodgrass, to mention only the more recent. But these authors have necessarily been preoccupied chiefly with describing the diversity of form among insects; discussions on function being correspondingly condensed. In the present work the emphasis is reversed. Structure is described only to an extent sufficient to make the physiological argument intelligible. Every anatomical peculiarity, every ecological specialization, has indeed its physiological counterpart. In that sense, anatomy, physiology and ecology are not separable. But regarded from the standpoint from which the present work is written, the endless modifications that are met with among insects are but illustrations of the general principles of their physiology, which it is the aim of this book to set forth. Completeness in such a work is not possible, or desirable; but an endeavour has been made to illustrate each physiological characteristic by a few concrete examples, and to include sufficient references to guide the student to the more important sources.

The physiology of insects is to some the handmaid of Economic Entomology. For although it is not the purpose of physiology to furnish directly the means of controlling insect pests, yet the rational application of measures of control—whether these be insecticides of one sort or another, or artificial interferences with the insect's environment—is often dependent on a knowledge of the physiology of the insect in question. Physiology may thus serve to rationalize existing procedures, or to discover the weak spots in the ecological armour of a species. A knowledge of the ecology of a species is always necessary to its effective control; its ecology can be properly understood only when its physiology is known.

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Chapter I

Development in the Egg

Cuticular membranes of the egg : the chorion—Most insects begin their independent life within an egg. When the egg is laid it is enclosed by two envelopes, the chorion and the vitelline membrane. The egg-shell or chorion is the product of the follicular cells in the ovary of the mother (p. 380). It consists typically of two layers ; a relatively thick endochorion, composed of a scleroprotein, ' chorionin '[43] and a very thin outer layer, the exochorion, applied like a varnish over the surface. Sometimes the exochorion is perhaps the final product of the follicular cells, but often it is indistinguishable from the secretion applied to the eggs by the accessory or colleterial glands in the oviducts. This secretion secures the eggs to the surface on which they are laid ; or it may serve to bind them together into a compact capsule or oötheca, as in Blattidae and Mantidae (p. 381).

The chorion may be thin and flexible, as in the eggs of such insects as *Tenebrio*, which are laid in protected situations ; usually it is more or less rigid, as in the exposed eggs of Lepidoptera, Hemiptera, &c. In Phasmidae, and likely enough in other groups, it is strengthened by the incorporation of lime in its substance.[28] In most insects it protects the egg from loss of water by evaporation ; but it is not completely impermeable to water, for insect eggs kept under too dry conditions may fail to hatch, in some cases because the embryo within is desiccated, in others because the chorion itself becomes too hard for the young insect to compass its escape.[8, 12]

Respiration may take place through the general surface of the egg when the chorion is thin ; but some eggs show structural adaptations which combine rigidity with the needs of respiration. In many Diptera the chorion is made up of little vertical columns, with the intervening spaces occupied at first by fluid, but later by air ; or extensive air-filled crypts may develop in the shell, widely spread or restricted to some limited area.[27] Similarly, in Locustids, respiration may take place through the general surface by means of superficial alveoli containing air, or at some region excavated to form a large reservoir.[9] The air sacs in the cap of Anoplura eggs, and the thin-walled evaginations of the chorion in Hemiptera, are likewise regarded as respiratory structures.[47]

The vitelline membrane originates from the cell wall of the ovum. In grasshoppers [40] and many other insects it remains a delicate structure and disappears as soon as development begins. In Tachinidae and other Diptera it is possibly of twofold origin—an inner oöplasmic zone, and an outer chorial zone, which is at first thick and viscous but later condenses to form a tough membrane [27] ; muscid eggs in moist conditions will continue their development within this sheath after the chorion has been stripped off.[48] In *Dytiscus*, also, the vitelline membrane is a very definite structure.[4]

The serosal cuticle—Later in development a layer of epithelium, the serosa, is formed beneath the vitelline membrane; and this may lay down another cuticle. In *Dytiscus* it is a stout laminated structure which has been called the 'secondary vitelline membrane'.[4] In the grasshopper *Melanoplus* it closely resembles the ordinary integument of the insect (p. 16); consisting of two layers, (i) a thin 'yellow cuticle', responsible for the impermeability of the egg membranes for salt ions,[23] secreted at 6 or 7 days after laying (at 25° C.), and (ii) a laminated white cuticle, composed largely of chitin (p. 19), apparently free from protein, secreted during the next week (Fig. 1). When complete this inner layer is generally thicker than the chorion and is responsible for the toughness of the grasshopper's egg.[40]

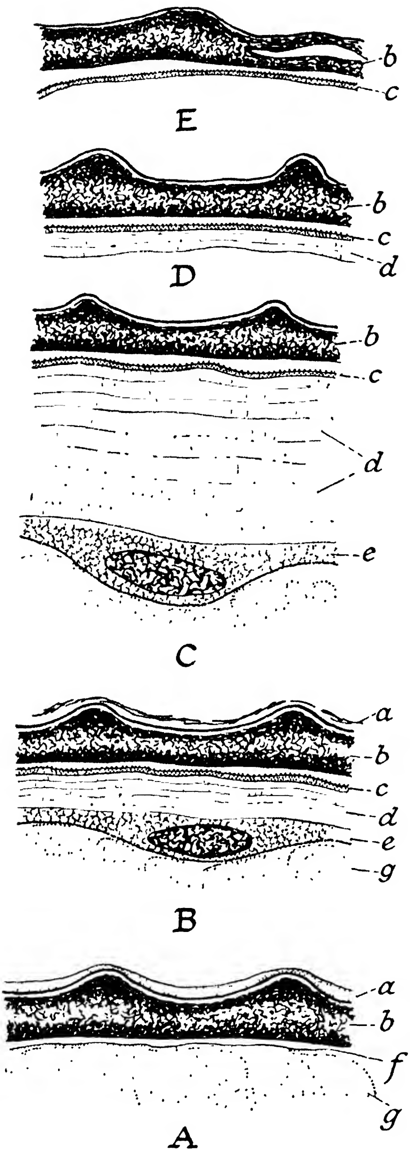

FIG. 1.—Egg membranes in *Melanoplus differentialis* (*after* SLIFER)

A, egg newly laid ; *B*, egg incubated 11 days at 25° C. (serosa has laid down the yellow cuticle and part of white cuticle) ; *C*, egg incubated 21 days (serosal cuticle fully formed) ; *D*, egg 3 days before hatching (serosal cuticle much reduced) ; *E*, egg ready to hatch (nothing remains but chorion and the yellow part of the serosal cuticle) ; *a*, temporary secretory layer over chorion ; *b*, chorion ; *c*, yellow cuticle ; *d*, white cuticle ; *e*, serosa ; *f*, vitelline membrane ; *g*, yolk.

Absorption of water—Eggs laid in moist surroundings may absorb water through their cuticular membranes. This was first noted by Rathke (1844) in the eggs of *Gryllotalpa* from damp soil, and in the aquatic Trichoptera ; it has since been observed in many other insects.[8, 32] In *Dytiscus* the egg, at first easily deformed by pressure, becomes tense and hard, increasing in length from 1·2 to 2·25 mm. ; the chorion splits and falls away and the egg is enclosed only in the vitelline membrane and serosal cuticle.[4] The egg of *Locusta migratoria*, which will develop normally only in moist soil, increases in weight from 6·3 mg. to 14 mg., the water content increasing from 52 to 82 per cent.[32] As the egg swells the chorion usually cracks and peals away ; but if kept moist it stretches and remains intact until hatching.[40] In the Capsid *Notostira*, water is absorbed from the tissues of the plant in which the egg is laid. Absorption begins about 55 hours after laying, at the same time as the formation of tissue becomes visible, and it reaches its maximum (a 75 per cent. increase) in about 160 hours (Fig. 2). In this case the chorion is resistant to distension ; the swelling forces off the preformed egg-cap, and the

egg is prevented from bursting only by the formation over the yolk of a cuticular plug laid down by the serosa and probably comparable with the serosal cuticle of other insects.[24] The eggs of Aleurodids are implanted into the leaves of plants by a stalk which bears a thin-walled terminal bladder; this is able to extract water from the plant and so make up for loss of water from the egg surface [46] (Fig. 3).

Special structures for the absorption of water probably occur in other insects also. Thus at the posterior pole of the egg of the grasshopper *Melanoplus* there is a small circular area in the 'yellow cuticle' secreted by a group of enlarged and modified serosal cells. The chorion over this region is more permeable than elsewhere. The whole structure is termed the 'hydropyle', since it is responsible for the uptake of water which begins as soon as the yellow cuticle is formed.[40] In all cases the absorption of water is probably an active process, and not due simply to osmosis [8]; in *Melanoplus*, for example, the uptake varies with metabolic activity irrespective of the membranes present.[42]

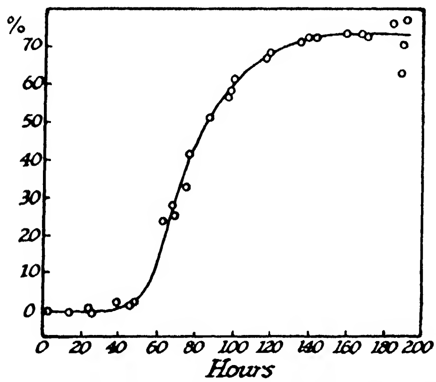

FIG. 2.—Increase of weight in eggs of *Notostira erratica* at 28° C. in contact with neutral water (*after* JOHNSON)

Ordinate: percentage increase in wet weight. Abscissa: hours after oviposition.

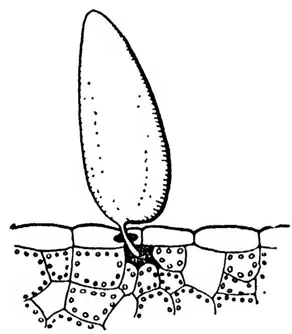

FIG. 3.—Egg of *Trialeurodes vaporarium* with the process, through which water is absorbed, inserted into a leaf and surrounded by cement (*after* WEBER)

In the eggs of endoparasitic Hymenoptera, laid in the body fluids of other insects, the egg-shell is reduced to an excessively delicate envelope which must be permeable to many substances in the blood of its host.

Early development—Fertilization of the egg takes place about the time of laying (p. 388). The fertilized nucleus lies embedded in the yolk, surrounded by cytoplasm which ramifies throughout the egg enclosing in its meshes the yolk spheres. At the surface of the egg there is a zone where the cytoplasm is rather denser and is free from yolk; this is the cortical layer, the 'Keimhautblastem' of Weismann.[48] The nucleus divides without segmentation of the plasma; the daughter nuclei are surrounded by islands of cytoplasm connected to one another and to the cortical layer by cytoplasmic strands. At first these cleavage divisions take place simultaneously throughout the egg; but after a definite number of mitoses, peculiar to each species, the nuclei begin to divide independently. The cleavage nuclei arrange themselves in a single layer enclosing a spherical or pyriform space in the centre of the egg (Fig. 4, A). As they divide they move towards the periphery of the egg and

the space which they surround expands and changes its form and becomes poorer in cytoplasm than the yolk elsewhere in the egg (Fig. 4, B). The advancing cells leave behind some of their number to form vitellophags or yolk nuclei. Ultimately the nuclei reach the cortical layer (Fig. 4, C). The cytoplasm, previously trailing like a tail behind each nucleus, is drawn in; the lateral divisions between the cells now appear; then their inner limits are formed, a basement membrane is laid down, and the formation of the blastoderm is complete. In a limited ventral region of the egg the cells of the blastoderm become higher and form a cylindrical epithelium; this is the germ band which is later differentiated to form the embryo; the remainder of the blastoderm, made up of flattened cells, forms the serosa.[14, 31]

It is not known what mechanism ensures the simultaneous mitosis of the nuclei in the early stages of segmentation; we shall see the same phenomenon

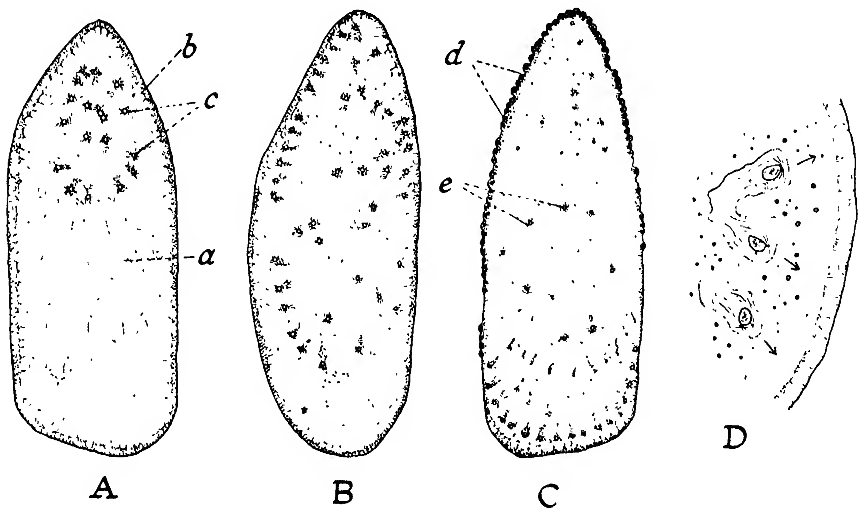

FIG. 4

A, B, and *C,* longitudinal sections of eggs of *Pieris,* showing migration of cleavage nuclei into the cortical layer (*after* EASTHAM). *A,* 6 hours after laying; *B,* 8 hours; *C,* 10 hours, nuclei of anterior pole have entered the cortical layer to form the blastoderm. *a,* yolk; *b,* cortical layer; *c,* cleavage nuclei; *d,* blastoderm; *e,* yolk cells. *D,* cleavage nuclei in *Calliphora* approaching the cortical layer (*after* STRASBURGER).

in the healing of wounds (p. 63). Nor is it known whether the nuclei migrate actively towards the surface or whether they are carried along passively by an outward streaming of the plasma.[31] In *Calliphora* the nuclei are always orientated so that the centriole is directed towards the surface of the egg, and after each cell division the inner daughter nucleus swings round so that the centriole looks outwards (Fig. 4, D); here the cells or nuclei are actively concerned.[41] But there is also evidence of an outward movement of the cytoplasm independent of the nuclei. In *Pieris* there are elongated flecks radiating in the untraversed regions of the yolk [14]; and in *Tenebrio,* granules in the yolk plasma wander through the reticulum ahead of the nuclei to reach the cortical layer. During the first four hours after the egg of *Tenebrio* is laid there is an outward movement of the plasma which causes a gradual thickening of the cortical layer, especially in the region of the presumptive germ band,

ventrally and laterally at the posterior pole; so that in this insect the future position of the germ band can be made visible by staining *before* the entry of nuclei.[17]

Organization—The nuclei in the egg contain the chromosomes bearing the genes which are responsible for many of the hereditary characters (p. 53); but the general form and organization of the embryo is controlled by agents located in the plasma at the periphery of the egg, quite remote from the dividing nuclei. The organism seems from the outset to be something to which the constituent nuclei are subservient, their function determined by their position in relation to the whole.

Two centres control the beginnings of organization in the insect egg. (i) At the posterior pole there is an *activation centre* (Bildungszentrum) which influences the peripheral zone of the egg in such a way that it can proceed to the subsequent stages of development. It does this probably by giving off a material substance which permeates the egg from behind forward. If this centre is eliminated at a very early stage by excision or exposure to ultra-violet light, cleavage and migration of nuclei occur as usual, but the resulting blastoderm is solely of the extra-embryonic type; no germ band is formed.[36] Elimination of other parts of the egg have not this effect; if the anterior parts are removed the embryo is merely smaller and the germ band displaced backwards (Fig. 6, B).[30] As the influence of this centre spreads forwards, during the early hours of development, increasingly large areas around the posterior pole must be excised or burned if the formation of the embryo is to be prevented. Within a few hours, although there is still no visible differentiation of any sort within the egg, the process of activation is complete (in *Tenebrio* there are at this time 64 cleavage nuclei [17]). A centre of this kind has been demonstrated in the dragon-fly *Platycnemis* (Fig. 5),[36] in the ant *Camponotus*,[30] in the weevil *Sitona*,[30] the bean beetle *Bruchus*,[6] and the mealworm *Tenebrio*.[17]

(ii) Towards the middle of the presumptive germ band, in a position corresponding with the future thorax of the embryo, there is a *differentiation centre*. . As soon as this centre has been induced to begin its activity, under the influence of the activation centre, it provides the focus from which all subsequent processes of development spread forwards and backwards. It is a part of the cortical plasma zone with no visible difference from any other part. But when the cleavage nuclei reach the surface and form the blastoderm they accumulate first in the region of this centre. Later, the blastoderm is thickest here; cell divisions are most numerous; and differentiation of body segments and appendages extends forwards and backwards from this point. The differentiation centre is in fact the place where visible differentiation of form begins in point of time and at which, later, the degree of differentiation at any moment is greatest. A centre of this type, which determines the future function of the nuclei that come into its sphere of influence, has been demonstrated in Hemiptera (*Pyrrhocoris* [36]), Odonata (*Platycnemis* [36]) (Fig. 5), Hymenoptera (*Camponotus* [30] and *Apis* [34]), Lepidoptera (*Ephestia* [35]), and Coleoptera (*Tenebrio* [17]). It is said to act, not by giving out a diffusible substance, but by initiating a contraction wave in the yolk system, spreading forwards and backwards and forcing the blastoderm cells, hitherto distributed uniformly, to fill the space that is left. Indeed, artificial depressions in the

yolk system caused by heat or by ultra-violet rays will bring about this directed movement of blastoderm cells to any desired part of the egg.[36]

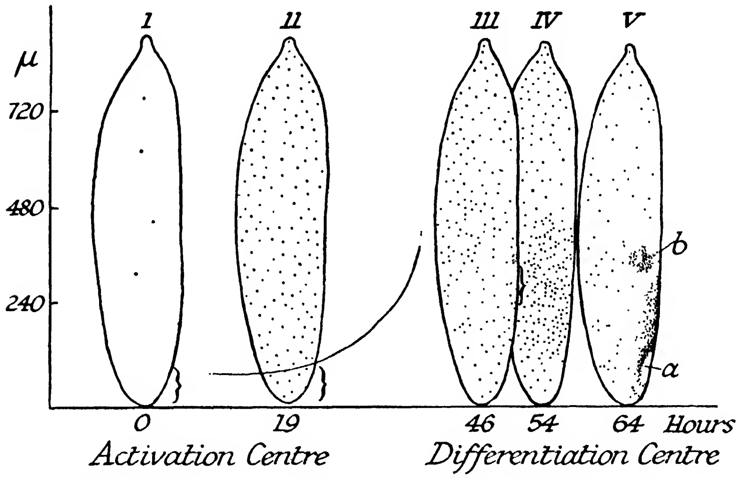

FIG. 5.—Organization in egg of *Platycnemis pennipes* (Odonata) (*after* SEIDEL)

Ordinate: length of egg in μ. Abscissa: time of development in hours at 21·5° C. (i) 0 hours, stage with 4 nuclei (position of activation centre indicated by bracket); (ii) 19 hours, stage with 256 nuclei. Influence from activation centre spreads forward as indicated by the curved line; (iii) 46 hours, cells accumulating first in region of differentiation centre indicated by bracket; (iv) 54 hours, germ band forming; (v) 64 hours, germ band fully formed: a, point where germ band sinks into the yolk; b, cephalic lobes forming.

Determination—Until the differentiation centre has performed its function, the egg is capable of extensive 'regulation'; that is, complete formation

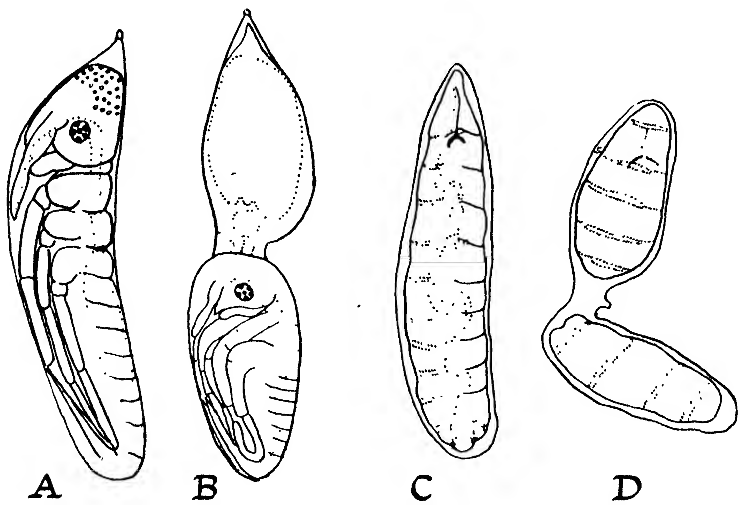

FIG. 6

A, *Platycnemis pennipes*; normal embryo shortly before hatching; *B*, dwarf embryo of the same age produced by ligaturing the egg in the middle at the stage with 4 cleavage nuclei (*after* SEIDEL); *C*, Muscid larva, normal embryo shortly before hatching; *D*, embryo of *Calliphora* ligatured at the stage with 16 nuclei, showing mosaic development (*from* SEIDEL, *after* REITH and PAULI).

from reduced material. If the egg of *Platycnemis* is ligated in the middle soon after laying, a differentiation centre appears in each half in a definite position in harmony with the diminished whole, so that double dwarfs are

developed.[36] In the egg of the honey-bee 12 hours after laying, the 'potency' to form all parts of the body is still so concentrated around the differentiation centre that about one-fifth of the entire egg can be removed at the anterior end and development of a complete dwarf embryo follows. As development proceeds, the 'potency' to form the different regions spreads outward over the egg; and in the bee, by 24 hours after laying, the prospective functions of all the main parts are finally determined.[34] The egg is then a 'mosaic' egg. If any part is eliminated, a corresponding region of the completed embryo is missing.

In the greenhouse cricket *Tachycines*, as in *Platycnemis* (Fig. 7), the embryonic germ is still capable of well-marked regulation at the time the egg is laid; and if the germ band is divided with a needle, many types of doubling, at first of the whole body and later of individual organs, can be produced.[25] The converse process, the fusion of eggs to form a single embryo, is seen in Phasmids. Senile *Carausius* females will lay eggs containing anything up to 10 oöcytes which fuse to form a compound egg. If fusion takes place early in development, regulation occurs and a normal insect results; if fusion takes place later, double monsters are produced, doubling being most frequent at the anterior end where differentiation first occurs.[10] Other Phasmid eggs may have two micropyles; in them there is no fusion where the embryos come in contact; each develops as far as it is able in the space available.[10]

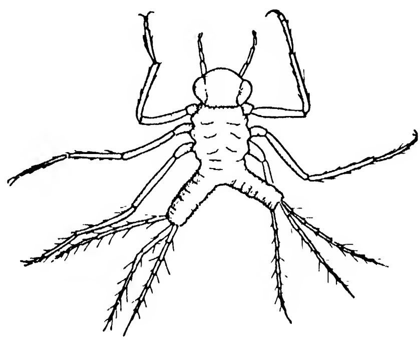

FIG. 7.—*Platycnemis pennipes*, showing doubling of the posterior part of the body as the result of dividing the prospective germ band by ultra-violet irradiation at the stage when the cells were beginning to collect around the differentiation centre (Fig. 5 (iii)) (*from a photograph by* SEIDEL)

Determination is completed at different times in different insects. In the bee, as we have seen, it is complete in 24 hours after laying[34]; in the bean beetle *Bruchus* in $6\frac{1}{2}$ hours[6]; while in Muscidae[29, 30] and in *Drosophila*[18] the egg has already reached the 'mosaic' state by the time it is laid. The process of determination, like the changes which precede it, is likewise independent of the nuclei in the egg. In *Platycnemis*, regulation is still possible in the late blastoderm stage.[36] In *Sitona*, the parts are fully determined by the time the blastoderm is formed.[30] In *Bruchus*, the posterior cytoplasmic regions of the egg are determined before the entrance of the cleavage nuclei, the more anterior parts shortly after the cleavage cells arrive.[6] Whereas in Muscid eggs, determination in the cortical plasma is complete before cleavage has begun (Fig. 6, D).[30] In all cases, determination of the main outline of the body takes place before the individual organs. When the process is complete it is possible to map out the prospective embryo by destroying localized spots on the egg surface with ultra-violet rays. In *Platycnemis* it has been found that at first the embryonic map delimited in this way covers almost the entire egg; as the cells congregate to form the embryonic rudiment the various zones become concentrated around the differentiation centre towards the posterior half of the egg.[36]

Determination of imaginal structure in the egg—In *Drosophila*, as in the Muscidae, the egg at the time of laying is a mosaic egg. Local injuries effected by ultra-violet light during the first 4 hours after laying cause local defects in the resulting larva. But the imaginal characters are unaffected; in respect to imaginal characters the egg is still capable of 'regulation'. But if the egg is irradiated similarly 7 hours after laying or later (between 4 and 7 hours the egg is so sensitive to treatment with ultra-violet light that experiments cannot be made during that period) localized defects are produced in the corresponding region of the adult epidermis without any visible effect during larval development.[18] Similar results can be obtained by puncturing the egg at different levels with a needle.[21] Indeed it can be shown that during this second developmental period a wave of 'determination' in respect of imaginal characters spreads backwards from the thorax until the egg becomes a mosaic egg in respect to adult structure also—just like the determinative change that spreads from the differentiation centre of *Platycnemis* and other 'regulation' eggs.

Later development—The later stages of embryonic development, the formation of the germ layers, the sinking of the embryo into the yolk and its subsequent revolution around the egg (blastokinesis), in the course of which the invaginated part of the blastoderm (the amnion) and the superficial part (the serosa) are absorbed into the body of the embryo, the growth of the embryo around the yolk, the formation and histological differentiation of the various organs and tissues, and the variations in these processes which occur in different groups of insects are fully described in the text-books.

Factors controlling the rate of development—The physiological control of the separate processes of later development has not been studied; but much information exists about the factors that determine the rate of development as a whole. The most important factor is temperature (p. 366): within the vital limits of each species development is accelerated by high temperatures and retarded by low, so that below a given temperature, peculiar to each species, no development takes place; growth is restored when the temperature is raised.

In many insects development is delayed by low humidity in the surrounding air.[8] At 20° C. the egg of *Sitona* (Col.) has an incubation period of 10½ days at a relative humidity of 100 per cent., 21 days at a humidity of 62 per cent.,[2] and in *Lucilia* there is a linear relation between saturation deficiency and the duration of egg development.[16] Presumably the delay under dry conditions is due to lack of water in the egg. For example, at the time of laying the egg of *Notostira* (Capsidae) does not contain enough water for complete development. If it is not given the opportunity of absorbing water (p. 3) embryonic development ceases, recommencing only when water is supplied.[24] Development is delayed from the same cause in the eggs of Aleurodids (p. 3) if laid on drying or wilting leaves,[46] and in the egg of the grasshopper if the 'hydropyle' (p. 3) is covered with material impermeable to water.[40]

Eggs of some insects can remain dormant for long periods in a desiccated state. Those of *Sminthurus* (Collembola), which hatch in moist conditions in 8 or 10 days, will shrivel and collapse if dried. They have been kept in this state for 271 days. When wetted they quickly become spherical again and hatch in 12 days or so.[20] Eggs of the South African locust, *Locusta pardalina*, which hatch in moist soil in about two weeks, have survived desiccation for

$3\frac{1}{2}$ years.[8] In such cases it is only at a restricted and usually an early stage of development that the eggs show this resistance.

Arrested development—This type of arrested development, controlled directly by external conditions and brought to an end when the temperature or water relations are favourable again, is sometimes termed 'quiescence'. But many insects, particularly those which pass the winter in the egg, undergo a spontaneous arrest of development which supervenes irrespective of the environmental conditions. For this state of dormancy Henneguy proposed the term 'diapause'. We shall discuss later the occurrence of this state in larval, pupal and adult life (pp. 67, 394); here we shall consider diapause only in relation to embryonic development. The arrest takes place at a different stage in different groups. Among Lepidoptera, almost no development occurs until the spring in *Zephyrus betulae*, &c., whereas the larva of *Argynnis paphia*, &c., is fully developed in the autumn [44]; *Orgyia* eggs cease developing when the germ band has formed and is beginning to give rise to mesoderm.[11]

Diapause in Bombyx mori—The classic example of diapause in the egg is afforded by the silkworm. Silkworm eggs laid in the autumn will not develop immediately even if kept warm; growth is completely arrested at an early stage. As was shown originally by Duclaux,[13] they will not hatch even in the spring if they have been kept warm (15–20° C.) throughout the winter; they will complete their development only if they have been exposed to a temperature around 0° C. for several months. Some races of *Bombyx mori* are single brooded or 'univoltine', so that every generation shows a prolonged period of arrest during embryonic development; other races are 'bivoltine' or even 'tetravoltine'; in these there are one or more uninterrupted generations during the summer before the winter generation of diapause eggs is produced.

The mechanism of diapause and the mode of action of low temperature in bringing it to an end (this 'reactivation' by cold is a characteristic of diapause at all stages of growth) will be discussed in conjunction with post-embryonic development (p. 67). But some indication of the kind of physiological factors that are at work is given by the study of voltinism in the silkworm. Voltinism is to some extent hereditary; but when the races are crossed, clear cut segregation does not occur. For the voltinism of the offspring is influenced by the temperature at which eggs of the preceding generation were incubated (eggs incubated at 25° C. tend to produce moths laying hibernating eggs; those incubated below 15° C. tend to produce non-hibernating eggs) and by the effect of temperature on the larva. The voltinism of the eggs seems in fact to be determined by some influence from the somatic cells of the mother. For a batch of eggs from a single female is generally uniform as regards voltinism; and if the ovaries of one race are transplanted into another race during the larval stage, the eggs from these ovaries always show the voltinism of their new host.[45]

Diapause in Melanoplus—There are all degrees of dormancy between a simple quiescence and a true diapause. The silkworm is an extreme example of the latter type; the grasshopper *Melanoplus differentialis* may be taken as an example of arrested development which is much more readily influenced by environmental factors.

In the winter generation of *Melanoplus* growth ceases when the embryo

is fully differentiated but quite small.[7, 39] It remains in this state without further cell divisions until the spring. Then growth is vigorously resumed; the embryo undergoes blastokinesis, moving around the lower end of the egg,

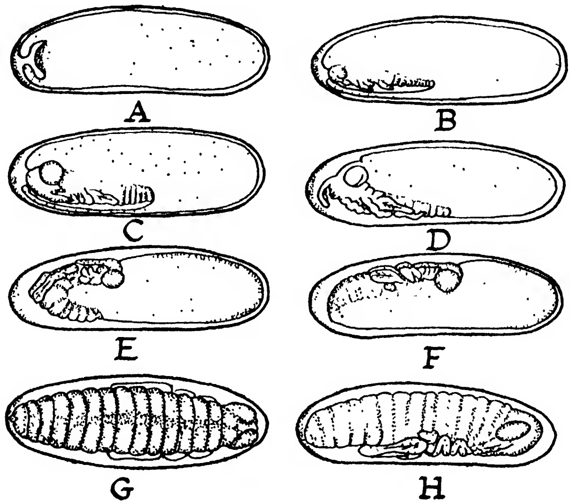

FIG. 8.—Embryonic development in *Melanoplus differentialis* (*after* BURKHOLDER)

A, appearance of germ band (5th day); *B*, embryo sunk into the yolk (10th day); the peak of oxygen consumption occurs at this stage; *C*, embryo in diapause; *D*, embryo beginning to revolve; amnio-serosal membranes have ruptured; *E*, embryo half revolved; *F*, early revolution completed; *G*, dorsal view, and *H*, lateral view after the dorsal line has fused and lateral rotation has taken place.

rotating on its long axis, and finally growing dorsally to enclose the remaining mass of yolk (Fig. 8).[39] These changes in growth activity are marked by changes in respiration (p. 344): there is a peak of oxygen uptake during the initial stage of growth (which occupies about three weeks at 25° C.), a long period of very low oxygen uptake during the diapause, and a rapid rise when growth is resumed (Fig. 9, B).

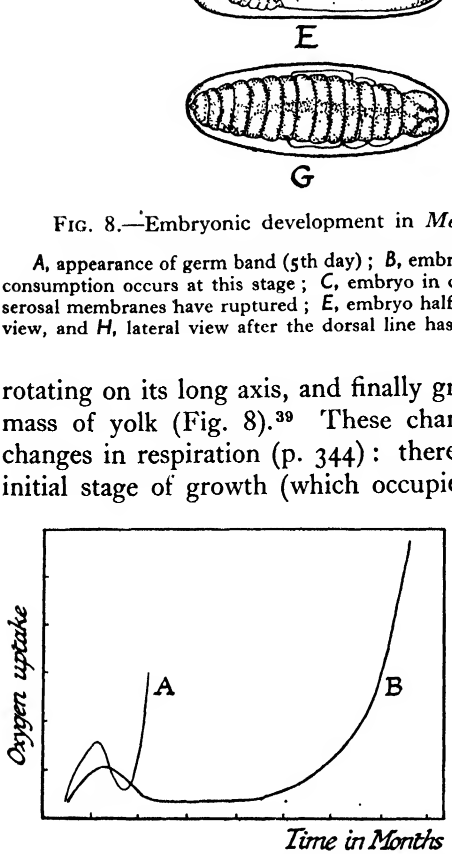

FIG. 9.—Oxygen uptake in eggs of *Melanoplus differentialis*

A, eggs at constant temperature of 27° C.; *B*, eggs kept at outdoor temperature and oxygen uptake estimated at that temperature (*after* BODINE).

This rhythm of development and arrest in the *Melanoplus* egg is not so fixed as that in *Bombyx mori*; for at any time during the winter a constant high temperature will initiate development, and the diapause can be prevented altogether if the eggs are kept at 25° C. from the time of laying. But an inborn rhythm probably exists in this insect also; for the longer is the egg exposed to cold in the winter, the greater is the rate of development upon returning to 25° C.; and once development has been resumed it cannot be arrested again by cold.[5] Moreover, *Melanoplus* eggs kept throughout at 25° C., which appear to have no diapause, still show an initial peak of oxygen uptake succeeded by a fall (Fig. 9, A); whereas in the eggs of *Chortophaga* (a grasshopper which

spends the winter as a nymph) the oxygen uptake rises steadily as development proceeds.[5]

Dormancy in mature embryo—Some insects complete their development in the egg before becoming dormant. That is so in the beetle *Timarcha tenebricosa*. The egg containing the fully developed larva may be kept at 15° C. for 6 months without hatching, and if the larva is extracted it soon dies. Whereas if kept at 5° C. for some time the larvae will survive extraction; and if exposed to severe frost for several months they all hatch simultaneously a few days after return to room temperature.[1] Mosquitos belonging to the genus *Aëdes* are another example of this type. The eggs containing fully formed larvae will survive in a dry state for several months (eggs of *Aëdes aegypti* have hatched after keeping for 40 days at 28° C. in air dried with sulphuric acid[8]); upon immersion in water many of them hatch within a few minutes.

Pleuropodia—In the mature embryo a pair of glandular organs is often present on the first abdominal segment. These were named 'pleuropodia' by Wheeler, who showed that they are homologous with the appendages of the first abdominal segment. They appear to be best developed in the less specialized groups (Blattidae, Mantidae and other Orthoptera, some Coleoptera); less so in other Coleoptera and Hemiptera; while in Hymenoptera and Lepidoptera they are vestigial or wanting.[22] Sometimes, as in *Belostoma* and *Ranatra* (Hem.),[22] they sink into the body wall, their distal ends prolonged into long thread-like structures forming a tuft projecting from the orifice (Fig. 10); sometimes, as in Orthoptera, they are stalked bodies projecting from the surface, made up of a single layer of very large cells.[33] They attain their greatest size just before the insect hatches, and then degenerate.

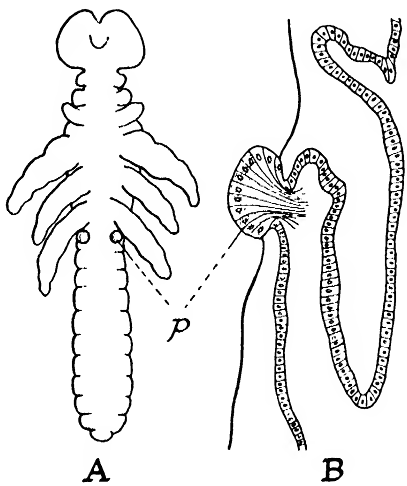

FIG. 10

A, isolated early embryo of *Ranatra fusca* to show position of pleuropodia; *B*, detail of pleuropodia as seen in sagittal section. *p*, pleuropodia (*after* HUSSEY).

The function of these organs may vary from one insect to another. In the viviparous Polyctenid *Hesperoctenes* their processes form a nutritive sheath which later secretes a protective cuticle (p. 398); in *Rhodnius* they are perhaps responsible for producing the iridescent cuticle which appears over embryo and serosa during blastokinesis [26]; and other secretory and excretory functions have been ascribed to them.[22, 33] In *Melanoplus* the inner white layer of the serosal cuticle (p. 2) is digested and dissolved shortly before hatching, the outer yellow cuticle being unchanged (Fig. 1, D, E). The enzymes responsible for this digestion are secreted by the pleuropodia; for if these are excised before digestion has occurred, the embryo develops normally but is unable to break through the cuticle, which remains thick and tough (Fig. 11).[40] This process is analogous to what happens in the insect cuticle during moulting (p. 26).

Hatching—When its development is complete the embryo is faced with the

problem of breaking the membranes and escaping from the egg. The main obstacles are the chorion, the vitelline membrane sometimes (in Muscids), and the serosal cuticle (in *Dytiscus*, Acridiids, and perhaps in other insects). In addition to these, many insects lay down a provisional cuticle which is later shed and replaced by a more substantial cuticle before hatching takes place. If this provisional or embryonic cuticle is laid down at a very early stage, as in the Aphidae,[19] it is a simple sac without appendages (perhaps this is not distinguishable from the serosal cuticle). If it is formed later, when the limbs are differentiated, each of these is invested in an unsegmented sheath, as in most hemimetabolic insects (Orthoptera, Hemiptera, Neuroptera, &c.) and in some holometabolic forms such as *Tenebrio* (Col.). Thus the embryo at the time of hatching is frequently enclosed by three sheaths.

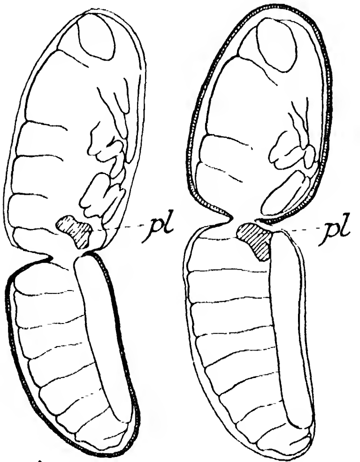

FIG. 11.—Diagram of an experiment to demonstrate the origin of the hatching enzyme in *Melanoplus* from the pleuropodia (*after* SLIFER)

Eggs with chorion removed are ligatured: *A*, behind pleuropodia; *B*, in front of pleuropodia. The white inner layer of the serosal cuticle is dissolved in the half containing the pleuropodia; the thin yellow outer layer is not attacked. *Pl*, pleuropodia.

Many insects are provided with cuticular structures, spines or blades, hard plates or eversible bladders, which are used to cut through the membranes of the egg or to force off a preformed egg-cap. These 'hatching spines' are developed on the embryonic cuticle when this is present (in Hemiptera, Neuroptera, Anoplura, &c.); when such a cuticle is absent, they occur upon the true skin of the larva and are thus retained throughout the first instar (in Nematocera, Aphaniptera, Carabids, Dytiscids, Chrysomelids, &c.).[15, 37]

The mechanism of hatching is pretty constant. The first sign that it is imminent is the appearance of pumping movements in the pharynx indicating that the insect is swallowing the amniotic fluid. This has been observed in *Agrion* (Odonata) and *Hydrobius* (Col.)[3] and in many other insects.[37] Swallowing continues until all the fluid is absorbed and the insect fills the shell completely. It may then rupture the chorion and the other membranes by simple muscular force (we have seen that the serosal cuticle may first be weakened by digestion of the inner parts (p. 11), or it may contract the posterior parts of its body and, by driving the blood forwards to the head, cause this to burst the shell. The hatching spines may serve to concentrate this pressure at one point of the egg (often the egg-cap, as in Pentatomidae) or they may be used deliberately to cut an opening in the chorion (as in Aphaniptera). The Lepidoptera simply gnaw their way out with the mandibles; the Muscidae tear a hole in the vitelline membrane with their mouth hooks.[37]

Often, when the amniotic fluid has been swallowed, air diffuses through the chorion and the insect may increase its bulk by swallowing this. Where the insect has to pass the narrow orifice that was closed by the egg-cap, it does so by peristaltic waves of muscular contraction passing forwards over the

body, squeezing out the head as a tense little vesicle (Fig. 12). During this process the embryonic cuticle may split and remain as a frill round the mouth of the shell (Anoplura ; Hemiptera) or the insect may leave the egg completely

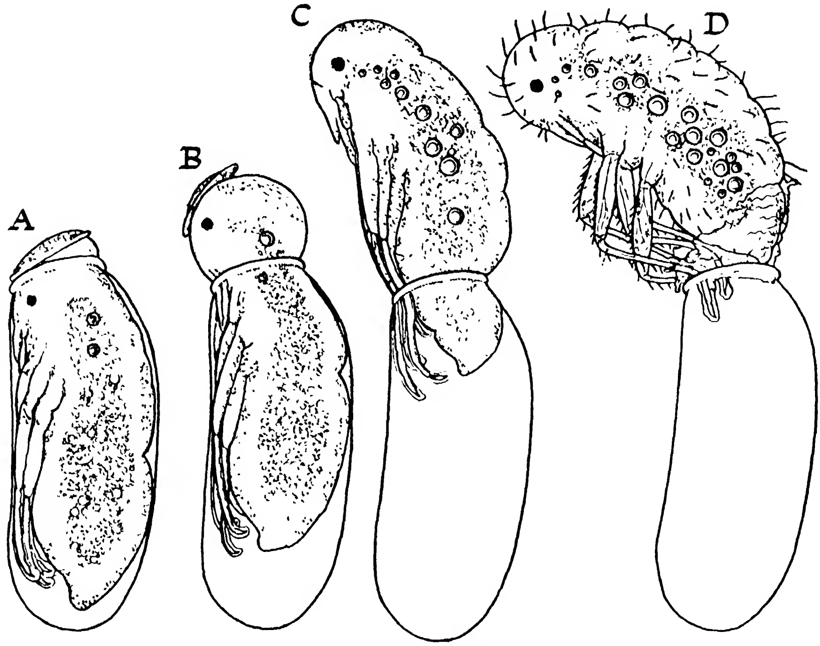

FIG. 12.—Hatching of *Cimex* from the egg (*after* SIKES and WIGGLESWORTH)

A, cap of the egg being forced off ; gut and abdomen showing waves of peristalsis towards the head ; a few air bubbles in the gut. *B*, active peristalsis continues ; head distended and bulging from the egg. *C*, larva, almost free from the egg, has started to swallow air vigorously. *D*, embryonic cuticle has split and slipped backwards, allowing spines to stand erect ; tracheae have filled with air ; larva swallowing air.

with this cuticle intact (Odonata ; aquatic Hemiptera ; Orthoptera). Thereupon it swallows air again, blowing itself up until this cuticle is forced to split ; and it may continue the process or, in aquatic forms, it may swallow water, until its skin has stretched and it has reached a size far greater than the egg from which it came.[37]

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Chapter II

The Integument

THE PHYSIOLOGY of growth in insects is so profoundly influenced by the properties of their integument that we must devote a chapter to this subject before returning to the further course of their development. As in all Arthropods the integument consists of a single layer of epidermal cells (in insects often called the ' hypodermis '), which secretes over the surface of the animal,

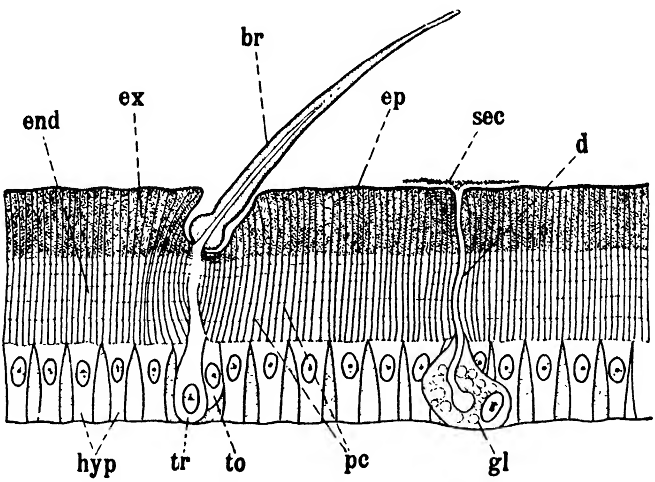


FIG. 13.—The insect cuticle: semischematic

br, hair or bristle; *d*, duct of dermal gland; *end*, endocuticle; *ep*, epicuticle; *ex*, exocuticle; *gl*, dermal gland; *hyp*, epidermis (hypodermis); *pc*, pore canals; *sec*, ' Sekretschicht '; *to*, tormogen or socket-forming cell; *tr*, trichogen or hair-forming cell.

and over all those invaginations of the ectoderm that arise from it—the buccal cavity and fore-gut, the hind-gut, the tracheae, the lower genital ducts and the multifarious glands that open upon the surface—a non-living cuticular membrane.

PROPERTIES OF THE CUTICLE

Structure of cuticle—This ' cuticle ' may be an excessively delicate pellicle, as in the terminal branches of the tracheal system and the intracellular cavities of certain glands, or a thick, dense, horny armour such as that which encases the thorax and appendages of many beetles. The characteristic

structure of the cuticle is best seen in the plates or sclerites on the dorsum of the abdomen as described, for example, in the larva of *Tenebrio*,[47] in *Gryllotalpa*,[22] in aquatic Hemiptera,[48] in queen termites,[1] and in the Reduviid bug *Rhodnius*.[73] It consists of three layers (Fig. 13): (i) an outer thin refractile membrane, 1μ or less in thickness, sometimes darkly pigmented, but often apparently colourless, called the '*epicuticle*' (the 'Grenzlamelle' or 'Grenzsaum' of German writers). (ii) Below this is a rigid layer, usually amber coloured, but sometimes almost black, which may compose anything from one-twelfth to one-half of the total thickness of the cuticle. This is the '*exocuticle*' (or 'primary cuticula,[66] 'Emailschicht',[6] 'Lackschicht',[53] or 'Pigmentschicht'[34]). (iii) Finally there is a thick colourless elastic layer, the '*endocuticle*' (or 'secondary cuticula',[66] 'Hauptlage' or 'Innenlage'), which makes up the greater part of the entire structure.

Pore canals—The epicuticle is variously folded and may bear innumerable little spicules or microtrichia, 'idiocuticular' structures.[37] Otherwise it is homogeneous. The endo- and exo-cuticle are traversed by numerous vertical lines. When seen in surface view these appear as minute dots, often arranged in polygonal fields, 20 or more together, separated by clear boundaries, corresponding to the limits of the epidermal cells.[22, 29, 47] These vertical lines were called 'pore-canals' by Leydig, and were regarded by him as filamentous processes of cytoplasm from the epidermal cells, around which the non-living substance of the cuticle was secreted. This interpretation is probably correct. It is supported by observations on *Leptinotarsa*,[66] *Gryllotalpa*, and other insects,[22] aquatic Hemiptera,[48] *Rhodnius*, *Tenebrio*, and *Periplaneta*.[73] As several authors have pointed out, it brings the structure of the arthropod cuticle into line with that of the bone or dentine of vertebrates. Some authors, on the other hand, accept the cytoplasmic origin of the 'pore-canals' but consider that they are later transformed into cuticular substance.[5, 26, 47] That may be so in some insects; but in many the patency of the canals can be readily demonstrated by cutting sections of fresh cuticle with the freezing microtome without fixing or staining, and drying them in air. The contents of the pore canals dry up and on mounting the sections the air-filled canals appear as black threads running a more or less convoluted course from the cells to the epicuticle, but not piercing the latter.

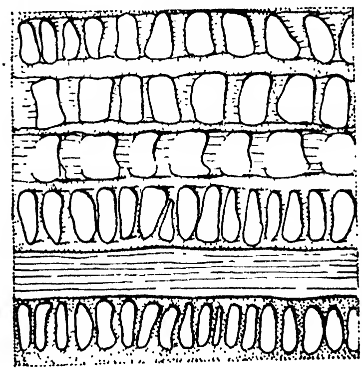


FIG. 14.—Section through cuticle of *Lucanus cervus*, showing the fibre bundles ('Balken') cut through at different angles (*from a photograph by* STEGEMANN)

Lamellae of endocuticle—The exocuticle usually shows no other structure, but the endocuticle is made up of horizontal lamellae becoming gradually thicker in the deeper layers. These, like the convolutions in the pore canals, are probably the result of a slight shifting of the epidermal cells as successive layers of cuticle are deposited. The lamination is sometimes regarded as a provision for bending and stretching, the lamellae sliding over one another slightly during the process.[1] In the horny parts of Carabidae and other

beetles, each of these lamellae is made up of parallel or anastomosing strands compressed laterally (the beams or 'Balken'[29]), embedded in a homogeneous matrix. The strands in superimposed layers lie more or less at right angles to one another (Fig. 14), and the pore canals as seen in surface view appear as stellate points among the criss-crossing 'Balken'. The strands are described by some as made up of bundles of fine fibrellae[6]; others regard them as having an alveolar or foam-like structure.[29] The former view agrees best with what is known of the micellar structure of the cuticle (p. 19). This arrangement of fibre bundles running in different directions in each superimposed lamella, is common in the skeletal structures of animals (in the placoid scales of Selachians, in the cornea and sclerotic of the eye[6]); in insects it is obvious only in Coleoptera; but it may really be more general, the separate elements being usually so small that they escape detection; for example, such a structure is described in *Gryllotalpa*.[22]

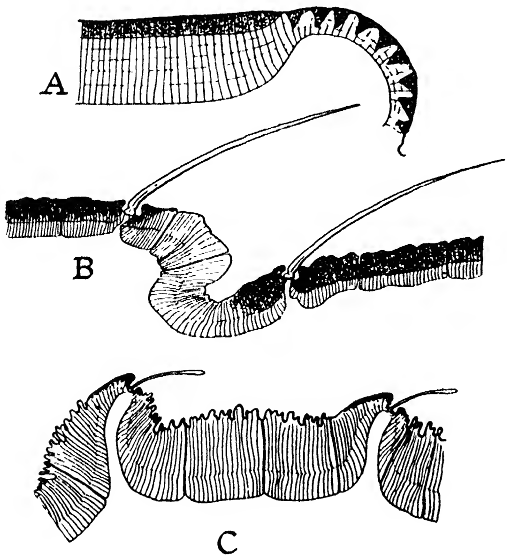


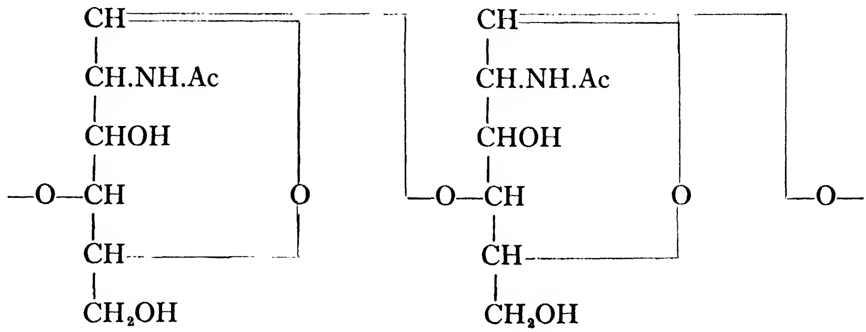
FIG. 15.—Examples of flexible and extensible cuticles

A, intersegmental membrane in *Tenebrio*; exocuticle broken up into wedge-shaped pieces (*after* PLOTNIKOW). *B*, intersegmental membrane in *Rhodnius* adult; exocuticle wanting. *C*, extensible cuticle of *Rhodnius* nymph; epicuticle folded, exocuticle absent except around bristles (*after* WIGGLESWORTH).

Modifications of cuticle structure—The foregoing account describes what may be regarded as the standard structure of the cuticle; there are many modifications to meet special needs. The cuticle is pierced in many places by the ducts of unicellular glands; the pore so formed is lined with epicuticle, and this extends inwards to cover the intracellular cavity of the gland.[52, 73] Then the cuticle is modified to provide receptor organs (p. 131), the typical example being the cuticular spine attached to the margins of a socket by a thin flexible membrane. At the folds or conjunctivae between the segments of the body or appendages the cuticle is likewise flexible; this is effected by modification of the rigid exocuticle, which may be broken up into wedges (as in the larva of *Tenebrio* (Fig. 15, A)[47] and in *Liogryllus*[22]), or may be absent altogether (in the majority of insects) (Fig. 15, B). In caterpillars, in queen termites, in the nymphal stages of *Rhodnius*, and in many other insects, the exocuticle is wanting over the greater part of the body: the endocuticle extends up to the highly folded epicuticle (Fig. 15, C). A cuticle of this type is exceedingly elastic. Nymphs of *Rhodnius* distend themselves with blood up to twelve times their original weight in a few minutes. The endocuticle stretches evenly over the entire surface of the abdomen and the complicated folds in the epicuticle are smoothed out.[73] As the meal is digested this process is reversible. Where it lines the larger cylindrical ducts, as in the salivary glands or the tracheae, the cuticle is thrown into folds which commonly run

a spiral course round the tube. Often the folds become filled with the cuticular substance to give rise to a spiral thread or ' taenidium '.

Chemical composition : chitin and protein—The best known constituent of the cuticle is the nitrogenous polysaccharide named ' chitin ' by Odier (1823) and later proved to be identical with the fungine which forms the cell wall of fungi. Chitin has the empirical formula $(C_8H_{13}O_5N)_x$; it is probably made up of a series of acetylated glucosamine residues linked thus [4] :



It is regarded, like cellulose, as being composed of long chains of this sort bound together by their secondary valencies to form elongated particles or micellae.[43] In cuticular hairs these micellae are probably set parallel with the axis and are responsible for their double refraction [14] ; they are responsible also for the characteristic interference patterns in the X-ray analysis of chitinous structures.[43]

Chitin is insoluble in water, alcohol, ether and other organic solvents, dilute acids, dilute and concentrated alkalis. It is soluble in concentrated mineral acids, being hydrolysed to lower saccharides, glucosamine, acetic acid, &c. When treated with caustic alkalis at a high temperature the acetyl groups are detached and the polysaccharide ' chitosan ' is produced. Chitosan gives a deep violet colour with iodine, and this provides the best test for chitin, van Wisselingh's test.[13, 72] It is performed as follows : the material is placed in a saturated solution of potassium hydroxide in a small tube, and this, together with a thermometer reading to 200° C., is immersed in a beaker of glycerine or paraffin, which is then heated to 150°–160° C. for 20 minutes (or longer if the material is not completely decolorized). The fragment is then transferred to a glass slide, washed in turn with 90 per cent. alcohol, 50 per cent. alcohol and water, and then flooded with 0·2 per cent. iodine in 1 per cent. sulphuric acid. An intense rose-violet colour is obtained. Chitosan prepared in this way is soluble in 3 per cent. acetic acid. It may be dissolved by warming in 10 per cent. sulphuric acid, and if left to stand at 70° C., spherites of chitosan-sulphate separate out.[37] Other tests are given by Campbell.[13]

Another colour test for chitin consists in treating the material with ' diaphanol ' (a solution of chlorine dioxide in glacial acetic acid) in the dark at room temperature for a day or more. This removes the non-chitinous constituents (' Inkrusten '), which bear much the same relation to chitin in cuticle as lignin does to cellulose in wood.[54] If the fragment of cuticle is then treated with the ordinary zinc chloride and iodine reagent used in testing for cellulose, it gives a violet colour.[31, 54] As a method of softening and bleaching hard pieces of cuticle this is a valuable procedure, but as a test for chitin it is not

entirely reliable.[13, 37] For example, the test often fails with the cuticle of caterpillars.[54]

Chitin is the main constituent of the endocuticle, of which it forms about 60 per cent. in *Periplaneta*. It is present also in the exocuticle to the extent of 22 per cent. in *Periplaneta*; but it is completely absent from the epicuticle.[13] The entire abdominal sclerites of *Periplaneta* contain from 35 to 38 per cent. of chitin,[13, 31] the wings 18 to 20 per cent.[61] The other principal constituent of the endocuticle, which is present also in the exocuticle but not the epicuticle, is protein: the inner parts of the cuticle readily give the protein colour tests, the biuret, Millon's and xanthoproteic reactions.[13, 37, 73]

'Cuticulin'—As was recognized by Haeckel, the two outer layers of the cuticle differ chemically from the endocuticle in being insoluble in concentrated sulphuric and hydrochloric acids in the cold. The substance which has these properties is present alone in the epicuticle; in the exocuticle it is incorporated with the chitin and protein. It is usually amber coloured, but may be mixed with the black pigment melanin. The chemical nature of this substance is unknown; the cast skins of bee larvae, which must be largely composed of it, are said to consist of a material containing 10·7 per cent. of nitrogen, and free from sulphur, not referable to any known group of organic compounds.[3] It has much in common with the cutin and suberin of plants.[37] It is soluble in hot 50 per cent. sodium hydroxide; when heated in a mixture of equal parts of saturated potassium chlorate and concentrated nitric acid, it fuses into masses soluble in ether, benzene, &c. ('cerinic acid test'). Fatty acids and probably cholesterol have been thought to be components, and the epicuticle is therefore described sometimes as a 'lipoid cuticula'.[37] But before this drastic treatment it is entirely insoluble in the ordinary organic solvents, so that the lipoid constituents cannot be present in a free state. The material is possibly a mixture of substances (as the 'cutin' of the plant cuticle is believed to be) but provisionally it may be termed 'cuticulin'.[73] In addition to cuticulin, the exocuticle is believed to contain polymerized carbohydrates other than chitin.[37, 54]

The cuticle lining the ectodermal invaginations has the same composition as that on the surface of the body. The larger branches of the tracheae contain chitin incorporated in the non-chitinous constituents [13]; the unicellular glands are lined only by the epicuticular cuticulin [73]; the finer branches of the tracheae and the tracheoles contain no chitin; their composition is unknown.

Lime in the cuticle—In a few aquatic insects lime is deposited in the cuticle. In some *Pericoma* (Psychodidae, Dipt.) larvae, when they occur in water rich in lime, this is deposited on cuticular processes on the body surface.[35] In the larva of *Sargus* and other Stratiomyids, the lime is in the form of warts laid down in shallow pits at the time of moulting, and intimately mixed with the organic substance of the cuticle. It may form about 75 per cent. of the integument. The mechanism of its deposition has not been elucidated, but most of the lime seems to come from within the body, probably from the Malpighian tubes, which become empty of lime at the time of moulting (p. 315).[44]

Permeability of the cuticle—The most important physical properties of the cuticle are its rigidity and its permeability. Enough has been said about its structure to indicate how rigidity and capacity for stretching are varied

according to the habits of the insect or the part of the body. The permeability of the cuticle to water is equally varied, and bears little relation to thickness or to rigidity. As a rule it is almost impermeable, and it is this fact which makes possible the terrestrial life of such small creatures. It is the epicuticle, and the cuticulin fraction of the exocuticle, which are responsible for this property [37]; and thus it is that such soft-skinned insects as the larvae of the clothes moth *Tineola* may be as resistant to desiccation as the heavily armoured forms.[42]

On the other hand, the epicuticle may be freely permeable. We shall see later (p. 27) that the cuticle is permeable to water until the time of moulting, although the epicuticle appears fully formed; the epicuticle lining the dermal glands must, of course, be permeable to the secreted products; the larger tracheal branches are probably impermeable, but the tracheoles allow water to pass through their walls (p. 185); and the cuticle lining the hind-gut and rectum in many insects allows water to be taken up through it (p. 270).

The cuticle of some aquatic insects, also, is relatively permeable; but there is much variation even among related forms. Thus, water and various vital dyes will diffuse through the cuticle of *Chironomus plumosus* larvae, but not through the cuticle of *C. gregarius* or *Corethra*. These differences again are not related to the thickness of the cuticle, which measures 7μ or more in *Chironomus*, $1{\cdot}5$–3μ in *Corethra*.[2] They are due to some difference in the cuticulin component; for after treatment with sodium hydroxide or 'diaphanol' they disappear. In mosquito larvae the general body surface seems to be completely impermeable, but the cuticle of the anal papillae is readily permeable to water and salts.[74] The same applies to the respiratory organs of some other aquatic insects,[32] and larvae of *Phlebotomus* can live only in contact with liquid water, which is absorbed by the skin.[63]

Secretions influencing permeability—Discharging upon the surface of the cuticle are numerous glands: wax glands, lac glands, silk glands, scent glands, glands secreting waste products, poisons or irritants, the moulting glands to be considered in the next section, and many others.[71] The secretion of certain of these glands, particularly those secreting wax, must play an important part in modifying the permeability of the cuticle. Thus *Trialeurodes* spreads wax over the body surface as a protective against loss of water.[70] A very striking example of this is seen also in the cockroach, on the cuticle of which there is an oily substance, presumably a product of the epidermal glands (*cf.* p. 331), which has a dramatic effect on the evaporation of water.[50] Fine droplets of water sprayed upon the cuticle become covered by a film of this substance and remain for hours without evaporating. But as soon as the temperature is raised to about 30° C. some change takes place in this film, presumably a change of phase such that the continuity of the film is destroyed and water is exposed at the surface, and the droplets of water immediately dry up. In the same way, the rate of evaporation from the insect shows a sudden increase when it is heated above 30° C. (Fig. 16). Evaporation from the cockroach can obviously take place through the cuticle.

Secretory layer of the cuticle—Some authors have regarded the epicuticle itself as formed by the outpouring of secretion from dermal glands.[22, 34, 53] This is certainly not the usual method of its formation (p. 24). But on the elytra of some beetles (Cicindelidae, Buprestidae) there is a thick layer, forming

as much as one third of the whole elytron, which is entirely wanting in the freshly emerged insect. This layer is heaped up around the pores of certain dermal glands, and it is absent from those beetles (e.g. the Mantichorini group of Cicindelids) in which glands are absent; it is therefore believed to be formed by their pouring out a viscous pigmented secretion, the 'Sekretschicht' or 'Sekretrelief'.[53] This layer provides the superficial sculpturing of the elytra, and granules of material embedded in it are responsible for the metallic colours of Buprestids, Cicindelids, &c.[59] The chemical nature of this secreted layer is unknown; it dissolves completely in 8 per cent. potassium hydroxide at 60° C. in the course of several days, leaving the usual layers of the cuticle exposed (Fig. 17).

Cuticle of the pupa—Pupae in which the appendages are free from the body, developing in protected situations, generally have a uniform thin cuticle. In pupae from exposed situations, the wings and appendages are often firmly

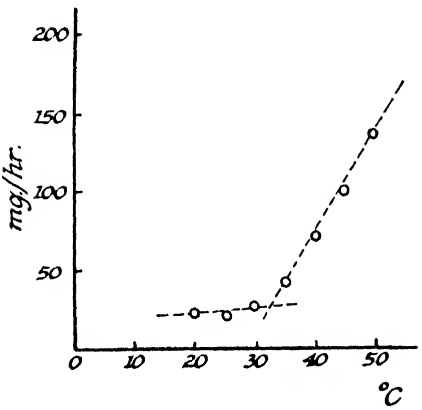


FIG. 16.—Rate of evaporation from *Periplaneta* in milligrams per hour at different temperatures

Saturation deficiency constant at 36 mm. Hg. (*after* RAMSAY).

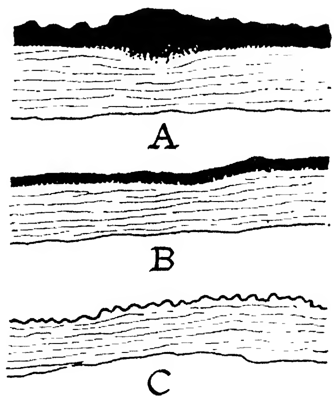


FIG. 17.—Cuticle of *Cicindela hybrida* showing the secretory layer (*after photographs by* STEGEMANN)

A, normal cuticle; B, after treating with 8% KOH at 60° C. for 36 hours (secretory layer partially dissolved); after 72 hours (secretory layer has disappeared).

stuck to the surface of the body by means of a fluid secretion poured over them at the time the last larval skin is shed. In *Leptinotarsa* (Chrysomelidae, Col.) this secretion is readily dissolved in water or alcohol in the early hours after pupation, so that the appendages become separated.[65] The extreme development along these lines is seen in the 'obtect' pupae of higher Lepidoptera, in which those surfaces of the appendages which are destined to form part of the outer sheath have a well-developed exocuticle, while the unexposed surfaces remain excessively delicate. At the last larval moult, when the pupa is exposed, the parts which form the sheath are cemented together by a secretion which soon hardens on exposure to the air, and only the surface outline of the appendages can be seen.

Another modification of the cuticle for the protection of the pupa occurs in the higher Diptera. When their larvae are full-grown, the epidermis adds to the soft existing cuticle a substance which slowly hardens and darkens to form a shell or puparium. Within this the very delicate pupa is formed.

Whether this substance is related chemically with the cuticulin of the ordinary cuticle is not known.

Metallic colours of the cuticle—In many insects the surface of the cuticle itself, or of the flattened scales which are articulated to it, show metallic or iridescent colours. The physical basis of these colours is quite different in different cases; in many it is unexplained. The metallic colours of Buprestid beetles are explained according to Walter's theory by the presence of granules of material in the surface secretion (' Sekretschicht ') of the elytra; granules which, like such dyes as eosin, both absorb and reflect, very powerfully, light of a particular wave length; metallic green elytra appearing red-brown by transmitted light.[23] But in most cases the colours result from some periodic structure in the cuticle which gives rise to interference colours. In at least one case, that of the Lamellicorn beetle *Sericea*, the iridescence is due

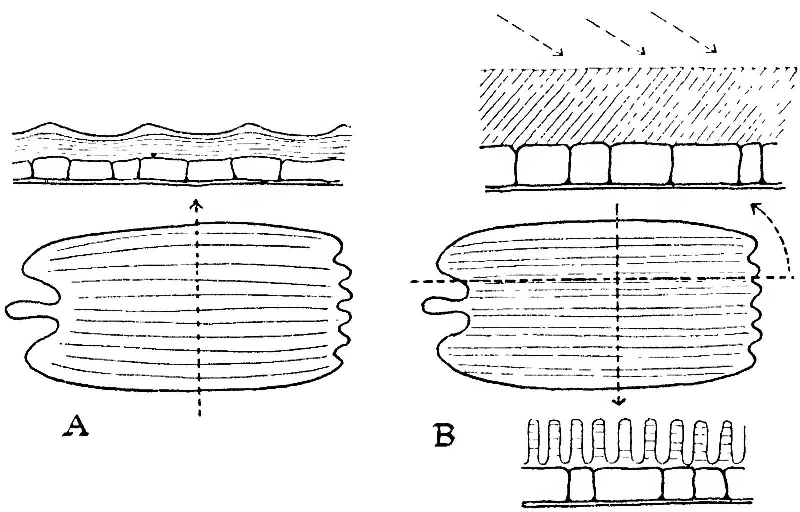


FIG. 18.—Two types of scales showing interference colours (schematic)

A, Urania type; transverse section above shows horizontal periodic structure in the upper lamina. *B, Morpho* type; longitudinal section above shows oblique periodic structure in the vertical ridges. Arrows above show direction of light to give interference. Below, transverse section showing ridges.

to diffraction by fine striae, 0·5–1·0μ deep and 1–1·5μ apart, evenly spread across the wing case; collodion impressions of the wing, bearing a cast of this structure, show the same iridescence as the wing itself.[41] In the majority of cases iridescence is due to interference by multiple thin films separated by material of slightly different refractive index.[7] Thin plates occur parallel to the surface in the Pentatomid *Calliphara*.[19] In the wing scales of Lepidoptera two main types are known: (i) The ' Urania type ' occurring in Zygaenids, Papilios, Lycaenids, in which either the upper or lower lamella of the scale is much thickened and made up of numerous thin superimposed plates (Fig. 18, A).[41, 45, 60] (ii) The ' Morpho type ', in which there is an oblique periodic lamination in the glass-like vertical ridges of the scales, so that the colour is visible only in the long axis of the scale and changes with the angle of vision (Fig. 18, B).[60] In the scales of the Diamond beetles *Entimus*, &c., oblique lamellae fill the interior of the scale, but are differently inclined in sharply defined areas, giving corresponding patches of colour.[41] The surface layer of

the cuticle in the highly iridescent Chrysididae (Hym.) is composed of little vertical columns each made up of superimposed lamellae which are probably responsible for the colours.[18] These periodic structures presumably arise spontaneously in the lifeless substance of the cuticle after it has been secreted; but that side of the problem has not been explored.

FORMATION AND SHEDDING OF THE CUTICLE

Since the cuticle is incapable of growth and, in the more rigid parts such as the head capsule or appendages, is incapable even of being stretched, it must be shed from time to time as the insect grows, and a new and larger cuticle laid down in its place. In the next chapter we shall consider the physiological factors which regulate this process of growth, but here we may describe those aspects of the subject which bear upon the properties of the integument.

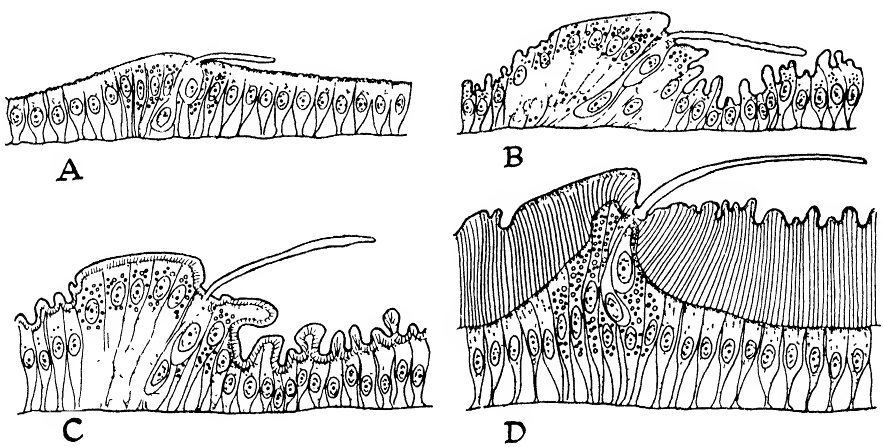


FIG. 19.—Longitudinal sections showing formation of new cuticle during moulting in 4th-stage nymph of *Rhodnius* (*after* WIGGLESWORTH)

A, 10 days after feeding; epicuticle just laid down. *B*, 11 days; epicuticle thrown into folds. *C*, 12 days; endocuticle being laid down. *D*, 15 days; just before moulting.

Formation of new cuticle—In the process of moulting or ecdysis the epidermal cells, which become greatly elongated at this time,[11, 66] separate themselves from the old cuticle, the cytoplasmic processes being withdrawn, it is said, from the pore canals,[66] dispose themselves to provide the proper form for the next instar, and proceed to lay down the new integument. They first secrete the epicuticle. This appears as a delicate smooth membrane continuous over the whole surface of the body (Fig. 19, A). It then seems to expand so that it is thrown into the folds characteristic of the ensuing instar (Fig. 19, B).[66, 73] Or the extensive folding which allows for growth during the instar may be secured by some cells being long and others short so as to form tongue-like projections or false villi.[11]

The inner, chitinous layers of the cuticle are then laid down (Fig. 19, B, C). The precise way in which this is achieved has excited much controversy. It doubtless varies in different insects, but a very common mechanism is that originally proposed by Leydig and accepted by many later authors,[5, 9, 22, 48, 73]

according to which the cuticular substance is secreted around the filiform outgrowths from the epidermal cells which later constitute the pore-canals. There is evidence that these vertical filaments extend into the substance of the epidermal cells [37, 73]; there is therefore very little difference from a physiological point of view between the secretion of cuticular material outside the cell and a transformation of cellular substance into cuticle.[6, 68] Doubtless both processes occur. Where the extracellular secretion is discharged in fluid form, there will be no indication of cell boundaries in the resulting cuticle, as in *Rhodnius* [73]; where the transformation takes place within the body of the cell, the cuticle will bear this impress and will be divided into little columns, as in the exocuticle of the elytra in many beetles.[37] The question whether the contents of the pore canals are later converted into cuticle has already been discussed (p. 17). During moulting and pupation in the silkworm, glycogen first appears in large amounts in the epidermal cells and then disappears as the new cuticle is laid down.[46]

A clear example of the formation of cuticle by the transformation of cell substance is seen in the formation of hairs and bristles. The microtrichia which are common on the epicuticle arise as little filaments from the surface of the epidermal cells.[64] Each large bristle arises by the outgrowth of a long cytoplasmic process from a large 'trichogen' cell. This process passes through an annular 'tormogen' cell that gives rise to the socket in which the bristle is articulated (Fig. 20).[20, 28, 73] In the later stages of its development chitin and cuticulin are laid down in the substance of this outgrowth to form the bristle.

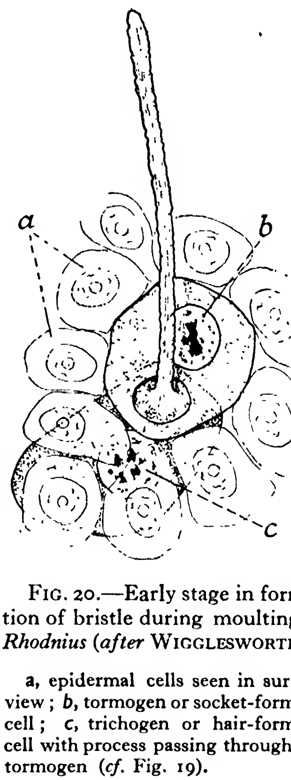


FIG. 20.—Early stage in formation of bristle during moulting of *Rhodnius* (*after* WIGGLESWORTH)

a, epidermal cells seen in surface view; b, tormogen or socket-forming cell; c, trichogen or hair-forming cell with process passing through the tormogen (*cf.* Fig. 19).

A rather special problem is presented by the criss-crossing strands or 'Balken' in the endocuticle of beetles. It is uncertain whether the adjacent epidermal cells co-operate in moulding them [29]; or whether they arise by the action of external stresses upon a homogeneous secretion from the cells.[6]

Moulting fluid—When the epidermal cells separate from the old cuticle and begin to secrete the new, the space between the two cuticles is occupied by a thin plasma. In the later stages of moulting this space is filled by an abundant fluid, the moulting or ecdysial fluid, first clearly demonstrated by Newport. There can be little doubt that much of this fluid, which extends also throughout the tracheal system, arises by exudation from the epidermal cells [8, 47, 48, 55, 76]; indeed, this has sometimes been considered its sole source.[11, 27] But the epidermis of the majority of insects contains numerous glands which become active only at the time of moulting and certainly contribute to the secretion of the fluid.

Such moulting glands were first described by Verson [67] in the silkworm, in which there are 15 pairs, each made up of a very large chief cell and two

cells connected with the duct. Glands of this type occur in all caterpillars and in saw-fly larvae (Tenthredinidae) [47]; in most insects there are widely scattered glands of similar form but much smaller (as in Chrysomelidae,[49, 66] aquatic Hemiptera,[48] the larva of *Dytiscus* and many other insects [27]). In the bug *Rhodnius* there are two distinct types of gland with the same general structure.[73] In the larvae of various Lepidoptera and Hymenoptera there is a gland in the main trunk of each trachea.[51]

An occasional third source of the moulting fluid is the secretion from the Malpighian tubes (p. 316). In the silkworm, the fluid contains crystals of oxalates and urates which come from these tubes and pass under the discarded cuticle of the rectum to reach the surface of the body.[47] These crystals are excluded if a ligature is tied round the anus,[57] an experiment which disproves the contention that during moulting the Verson glands assume an excretory function.[67]

Function of the moulting fluid—The chief function of the moulting fluid is to digest and dissolve the old endocuticle. The greater part of the old cuticle may be dissolved in this way; in the larva of *Tenebrio* and the pupa of *Papilio*, for instance, only the outer pigmented layer, the exocuticle, and a trace of the endocuticle remain.[47] In *Rhodnius* about 86 per cent. of the cuticle of the abdomen (from which an exocuticle is mostly wanting) is digested, and what is finally shed is a thin membrane consisting of little but the epicuticle (Fig. 21). The fluid, which is neutral in reaction, must in fact contain enzymes

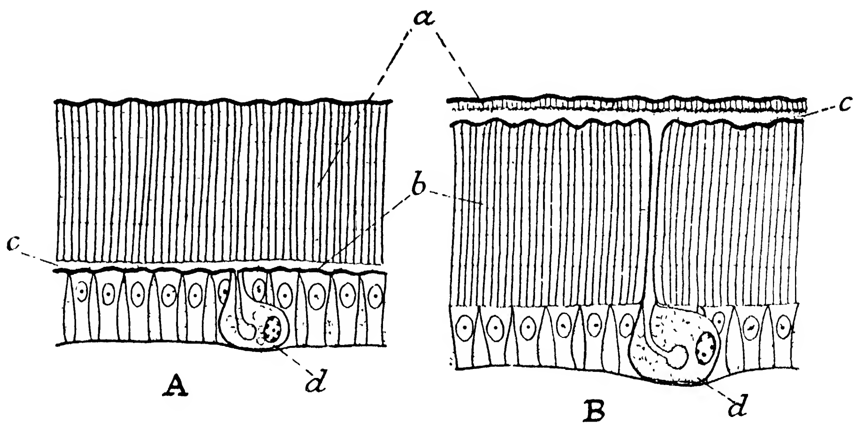


FIG. 21.—Moulting of a cuticle in which an exocuticle is wanting (*modified after* WIGGLESWORTH)

A, new epicuticle formed; digestion of old endocuticle scarcely begun. *B*, digestion and absorption of old endocuticle almost complete. *a*, old cuticle; *b*, new cuticle; *c*, moulting fluid; *d*, moulting gland.

capable of digesting chitin and protein; but it has no action on cuticulin: the exocuticle is unaffected and so are the delicate epicuticular linings of the dermal glands.[73] In *Leptinotarsa*, in which the same process of digestion is described, the enzymes have been thought to be secreted by the epidermal cells.[66] But at the time the process of solution is most active (in the last few days before the skin is shed) the epidermal cells are covered by the new cuticle, and it is highly improbable that they could secrete such enzymes through it. It is more likely that the enzymes are produced by the dermal moulting glands, which become excessively vacuolated in the last few days of

moulting. They are still full of vacuoles after moulting is accomplished; the remaining secretion, when it is discharged, forms a solid plug which blocks the duct in the Verson glands of caterpillars [55]; in *Rhodnius* it is poured out around the mouth of the duct a few hours after moulting, and there forms a little dry patch, apparently functionless (Fig. 13, *sec.*).[73]

In some insects, such as the larva of *Calliphora* [27] and the pupae of many Lepidoptera and Coleoptera,[47] dermal moulting glands are said to be absent. Perhaps this may be ascribed in some cases to the cuticle being so thin that digestion is not necessary; in others the glands may have been overlooked. If the digestion of the old cuticle were to be completed before the new cuticle is formed, and this may perhaps happen in some insects, the epidermal cells might themselves be responsible for the digestion process. That the epidermis can dissolve endocuticle is seen in the ptilinum of Muscid flies. When these emerge the endocuticle of the ptilinum is as thick as the other parts of the head capsule; but 14 days later nothing remains of it but the epicuticle with its spicules (Fig. 22).[39] And if a partial, localized, pupation is induced experimentally in caterpillars (p. 38), solution of the endocuticle by exuvial fluid may take place even though there is no chance of the secretion from the Verson glands reaching the area of cuticle in question.[36] Normally, as we have seen, much of the new cuticle is laid down before the old is digested; and it is probably owing to the epicuticle being laid down upon its surface that this new cuticle is protected from digestion.

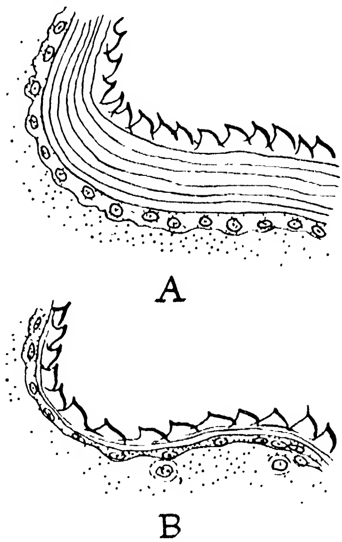


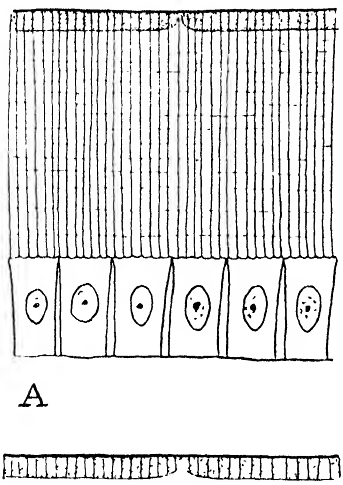
Fig. 22.—Section through ptilinum of *Calliphora*

A, newly emerged imago; *B*, 3 days after emergence, showing absorption of the inner layers of the cuticle (*after* Laing).

Absorption of moulting fluid—It is commonly believed that the principal function of the moulting fluid is to serve as a lubricant when the insect slides out of its skin. This moist layer will certainly keep the old cuticle soft and supple [8]; but by the time it is cast off almost all the moulting fluid has disappeared; the surface of the skin is dry, the old cuticle very nearly so. This cannot, therefore, be regarded as its most important function.

Almost all the moulting fluid, and the products of digestion from the cuticle, are in fact absorbed into the body of the insect before moulting takes place. In the aquatic pupae of Nematocera and the nymphs of May-flies a film of air appears beneath the cuticle just before emergence,[15] probably as the result of absorption of the moulting fluid. In the silkworm much of the fluid is swallowed by the mouth [69]; but in most insects it is probably taken up through the general surface of the body. As can be proved by injecting vital dyes between the two cuticles, that is certainly so in *Rhodnius*.[73] Evidently the cuticle is still permeable to water at this stage; indeed, if the old cuticle is stripped off, the insect rapidly dries up; and if adult flies, *Calliphora*, are removed from the pupa one day before emergence and immersed in water they swell by osmosis and the wings expand.[17]

Ecdysial line—It is often claimed that moulting is a process of excretion which serves to eliminate waste products. Obviously this cannot be true of chitin, the greater part of which is reabsorbed into the body. It might be true of the indigestible cuticulin, though, as we shall see later, there are objections to this also (p. 40). But the digestion of the endocuticle has another purpose besides the conservation of its substance. For in the young stages of all insects there is an 'ecdysial line' or 'Häutungsnaht', along which the cuticle splits most readily at moulting. It is usually a T-shaped line on head and thorax [16, 25] which appears white in pigmented insects because here the exocuticle is wanting and the endocuticle extends up to the epicuticle (Fig. 23). Consequently, when the endocuticle has been dissolved the ecdysial line constitutes a line of weakness where the slightest pressure will cause the cuticle to rupture.[47]



A

B

FIG. 23

A, transverse section of cuticle on dorsum of thorax of *Tenebrio* larva, showing the line of weakness or ecdysial line in the exocuticle (*after* PLOTNIKOW); B, the same after digestion of the endocuticle.

Mechanism of moulting—The mechanism by which the insect escapes from its cuticle and expands its wings and body has often been described. A very full and clear description of the process during the final moult of an Agrionid dragon-fly, which illustrates the principles common to most insects, is given by Brocher.[10] Like the insect hatching from the egg, the moulting insect contracts the abdomen and drives the body fluid into the head and thorax. It commonly assists this process, as was first shown by Jousset de Bellesme, by swallowing air [33] or, in aquatic insects, by swallowing water; if the crop of the cockroach is opened to the air the insect cannot moult, though it may remain alive a week or more.[16] The pressure so created splits the cuticle along the ecdysial line and the insect slowly draws itself out, often aided by gravity—for many insects hang head downwards while they moult. The linings of the ducts and glands [47] and of the tracheal branches down to a diameter of 3–5μ [8] come away with the old skin.

Sometimes there are special structures to aid the splitting of the skin, analogous to those concerned in hatching from the egg: Acridiids have an extrusible bladder in the neck [38]; the aquatic Hemipteron *Limnotrechus* has a similar blood-filled vesicle which is extruded beneath the scutellum.[16] The most elaborate is the ptilinum of Muscid flies; this consists of a bladder attached to the margins of the frontal cleft, everted by the blood pressure, as suggested by Réaumur (1738), and retracted by a special temporary musculature.[39]

Expansion of new cuticle—Escaped from the old skin, the insect, whose new cuticle is still soft and pliant, swallows air (or water) vigorously again and increases its bulk like the insect newly hatched from the egg. The muscles of its body wall remain in a state of continuous contraction so that the pressure of the blood, which is normally not much removed from atmospheric pressure,

is kept at a high level. In the newly moulted imago of *Aeschna* it is kept at about 75 mm. of water for several hours [56] until the cuticle is hard. If at this stage the gut is punctured, the insect collapses like a pricked balloon.[16] The pressure so maintained serves to expand the wings and other parts that were crumpled under the old skin. If the wings are punctured blood drips from them and they cannot expand.[10] Nor will they expand if the insect is narcotized with ether; though they will do so if the pressure is artificially increased by compression with the finger.[10] Perhaps the eating of the cast skin, a common habit among insects, helps to maintain the increased bulk.[16] Fraenkel [17] has described some ingenious experiments which demonstrate this mechanism of wing expansion. The blow-fly *Calliphora* increases its volume 128 per cent. by swallowing air. If it is allowed to swallow a little air and is then placed in carbon dioxide, this diffuses in with great rapidity (see p. 196) and raises the gaseous pressure so that the wings expand in a few seconds. The same instantaneous expansion takes place if the fly, after swallowing a little air, is exposed to a vacuum of 200 mm. of mercury.

Hardening and darkening of cuticle—The cuticle of the newly moulted insect is generally colourless, always quite soft. During the next hour or so it hardens and darkens. This is no simple effect of contact with the air as it is sometimes thought to be; for it fails to occur if the insect is killed immediately after moulting [30]; and if a part of the new cuticle is exposed by removal of the old cuticle 24 or 48 hours before moulting, although it appears fully formed it neither hardens nor darkens until moulting has taken place.[73] In *Calliphora*, expansion of the wings is generally complete in 20 to 30 minutes after emergence, darkening and hardening in 1 to 2 hours. But flies forced to keep digging through the soil for as long as 7 hours still remain pale and soft. Inflation, hardening and darkening occur as usual when they become free. Clearly these chemical processes are initiated by some nervous mechanism.[17]

Little is known of the actual chemistry of hardening; probably (like blackening) it is an oxidative change in the existing secretion, catalysed by oxidizing enzymes, rather than an impregnation with other substances; for neither hardening nor darkening takes place normally in an atmosphere of nitrogen.[24] These changes involve, of course, only the epicuticle and exocuticle. It is interesting to note that they may occur in an epicuticle separated from the epidermal cells by a considerable thickness of endocuticle. Perhaps it is a function of the pore canals to enable the cells to exert this action at a distance.[73]

Loss of water during moulting—Since the new cuticle is readily permeable to water almost up to the time of moulting, the question arises whether it remains permeable after moulting has occurred; that is, whether moulting involves a serious loss of water. What few data exist suggest that it does not. The dragon-fly *Aeschna* may show a 50 per cent. loss of weight during the first 24 hours after emergence [56]; but dragon-flies are known to discharge from the anus many clear drops of water as soon as expansion is complete [10, 21]; they probably eliminate water swallowed by the nymph before moulting. In the mealworm the conservation of water is so complete that moulting makes no measurable difference to the rate of evaporation [12]; in this insect there is no sign of any moulting fluid when the skin is cast.[76] In *Rhodnius*, weighing

about 112 mg., the water lost with the old skin is about 1·1 mg., the skin itself about 1·8 mg. During the next 48 hours, especially during the first 12 hours after moulting, the rate of water loss is increased above the normal; but the

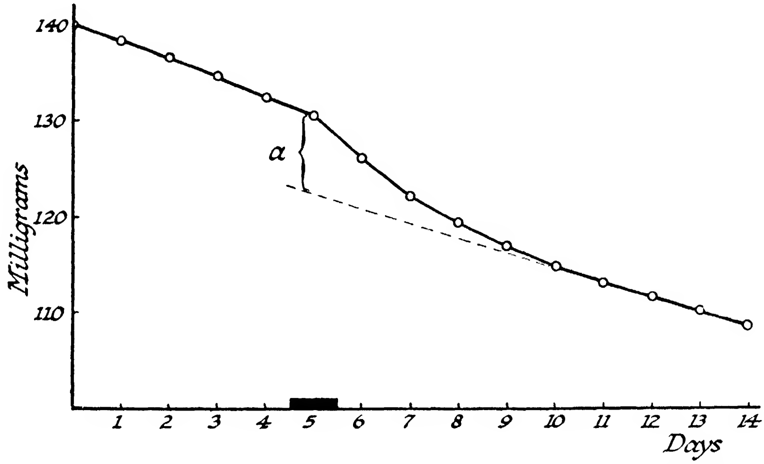


FIG. 24.—Loss of water during the moulting of *Rhodnius* 5th instar nymph (*after* WIGGLESWORTH and GILLETT)

Ordinate: body weight. Abscissa: days. The thickening of the base line indicates the day of moulting. a shows the extra loss of water due to moulting. (The weight of the cast cuticle has been added to the body weight after moulting.)

total extra loss associated with moulting is equivalent to no more than the normal insect loses in about 4 days (Fig. 24).[75] On the other hand, at the time of emergence from the pupa the skin of the beetle *Popillia* is quite moist and the insect loses at this time about one third of its weight.[40]

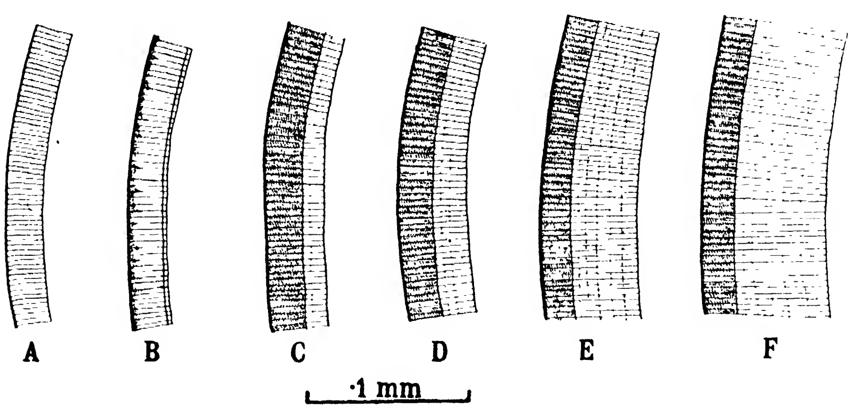


FIG. 25.—Deposition of cuticle after moulting. Transverse sections of cuticle of head of adult *Rhodnius*

Shading indicates impregnation with the amber-coloured cuticulin. *A*, immediately after moulting; *B*, 24 hours after moulting; *C*, 2 days; *D*, 4 days; *E*, 10 days; *F*, 1 month after moulting. (*After* WIGGLESWORTH and GILLETT.)

Thus the impermeability of the cuticle to water in these terrestrial insects is almost fully established by the time the skin is cast. But in some aquatic insects it seems to remain permeable longer. For the increase in volume of

aquatic Hemiptera after hatching can be prevented by immersion in 0·8 per cent. sodium chloride, and this procedure will even restore to its initial volume a nymph hatching into pure water [48]; and besides the water that is swallowed after ecdysis in *Notonecta*, *Gerris*, &c., much seems to be taken up through the cuticle by osmosis.[62]

There is no increased evaporation before moulting, although the old cuticle may be becoming excessively thin. This demonstrates again that it is the outermost layers of the cuticle which are responsible for its impermeability. The same can be shown after moulting. For although the impermeability of the new cuticle is established almost at once, nearly the whole of the endocuticle is yet to be laid down. In *Rhodnius* this process requires at least 3 weeks (Fig. 25) [75]; and in the elytra of Carabids the laminated structure is not complete 14 days after emergence.[58]

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Chapter III

Growth

MOULTING

GROWTH IN the animal body is always more or less cyclical, periods of comparative rest alternating with periods of activity. But in no group is this so evident as in the insects, in which development is punctuated by a series of moults or ecdyses, each preceded by a period of active growth and followed by a period in which true growth may be entirely absent.

Growth ratios—Moulting is primarily a mechanism of growth, conditioned by the properties of the cuticle. In many insects the amount of growth which is achieved at each moult is predictable from certain empirical laws. It was shown by Dyar many years ago that the head capsule of caterpillars grows in geometrical progression, increasing in width at each moult by a ratio (usually about 1.4) which is constant for a given species. This rule applies to many parts of the body, so that when the number of the instar is plotted against the logarithm of some measurement on the insect, a straight line is generally obtained (Fig. 26).[188] It is sometimes possible, by the help of this rule, to deduce from incomplete series of cast skins the actual number of ecdyses—as in *Haematopota* (Tabanidae), the pharynx of which shows a very constant ratio of growth of 1.29.[37] In other species, such as *Heliothis* (Lep.) [67] and *Popillia* (Col.) [2] there are so many departures from Dyar's rule that it cannot be used for corroborating the number of instars.

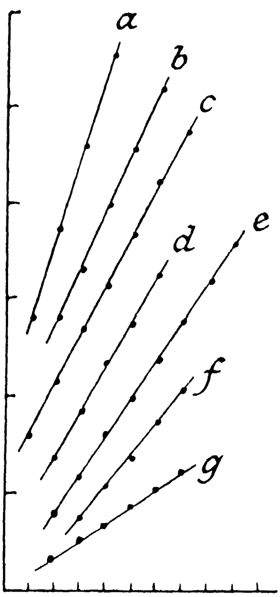

FIG. 26.—Chart illustrating Dyar's law (*after* TEISSIER)

Ordinate: logarithm of linear measurement. Abscissa: number of instar. *a*, *Bombyx mori*, ratio (r) = 1·91; *b*, *Philosamia ricini*, r = 1·52; *c*, *Crambus mutabilis*, r = 1·44; *d*, *Chaetopletus vestitus*, r = 1·40; *e*, *Sphodromantis bioculata*, r = 1·30; *f*, *Cladius isomerus*, r = 1·50; *g*, *Tenebrio molitor*, r = 1·12.

Another empirical law of growth is known as Przibram's rule. According to this the weight is doubled during each instar, and at each moult all linear dimensions are increased by the ratio 1·26 or $\sqrt[3]{2}$. This rule was deduced from measurements on *Sphodromantis*, which agree fairly well with these figures [154]; and the hypothetical explanation of the rule, that during each stage every cell in the body divides once and grows to its original size, is supported by the observation that in this insect there are in every stage an equal number of nuclei per unit area of cuticle.[186] *Dixippus* also moults when

its weight is about doubled (Fig. 27, A).[55] In various other insects, while growth in weight is more or less continuous, growth in length of the rigid parts of the cuticle is discontinuous; these often show a 25 per cent. increase at each moult, in conformity with Przibram's rule.[187] A peculiar form of discontinuous growth is shown by aquatic insects such as *Notonecta*, in which the weight may be abruptly doubled at moulting by the water swallowed or absorbed through the integument (Fig. 27, B).[187]

On the other hand, agreement with this rule is often so inexact that it becomes of no practical value, as in *Lymantria*,[76] *Melanoplus*,[87] *Locusta*,[98] *Tenodera* (Mantidae),[153] *Popillia*.[122] It is of doubtful value, also, where the weight increases several times at each moult; in which case it is supposed that such increase is always by some power of two.[15, 154] And the simple histological basis of this rule has had to be abandoned, because in Muscidae and other flies, the rigid parts of which show fair agreement with the numerical rule,[3] the larval cells do not divide after the embryonic period; they grow in

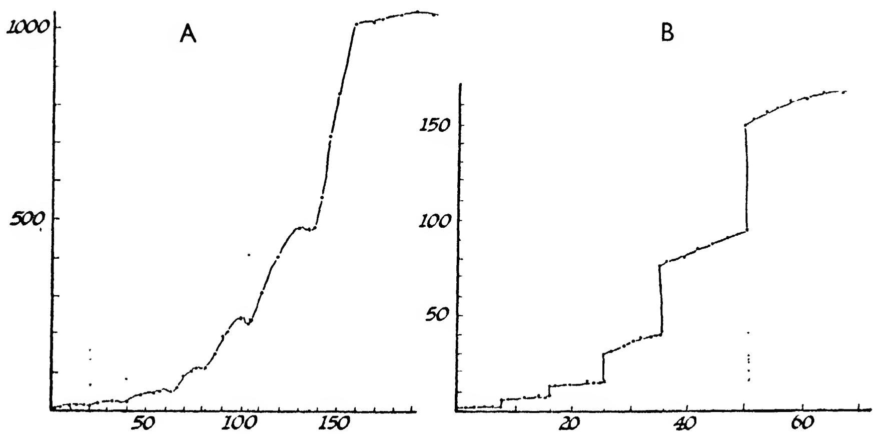

FIG. 27.—Changes in weight during growth (*after* TEISSIER)

A, *Dixippus morosus*; B, *Notonecta glauca*. Ordinates: weight in milligrams. Abscissae: time in days. The vertical dotted lines show the times when moulting occurred.

size only [141]; and in other insects, as we shall see, there is much cellular breakdown and reconstruction at each moult, no simple dichotomous division.

Allometric or Heterogonic growth—That the dimensions of a part of the body should increase at each moult by the same ratio as the body as a whole, which is what Przibram's rule implies, presupposes the occurrence of 'harmonic growth'. Whereas growth in insects, as in so many animals, is generally 'disharmonic', 'heterogonic' or 'allometric'—the parts growing at rates peculiar to themselves, higher or lower than the growth rate of the body as a whole.[93] This law is expressed by the formula $x = ky^a$ (where x is the dimension of the whole, y the dimension of the part, a the 'growth coefficient', and k another constant). That is, the logarithm of the dimension of the part is proportional to the logarithm of the dimension of the whole, so that when these measurements are plotted on a double logarithmic grid, a straight line is obtained (Fig. 28).

This type of growth may supervene late in development; in many insects it occurs chiefly during metamorphosis: the intermediate steps escape detec-

tion and it is the limitation of the total amount of growth achieved which is regulated in accordance with the formula. Moreover, this excess capacity for growth or 'growth potential' is often distributed unequally throughout an organ, and falls off in each direction from a centre of maximum growth. Thus in stag beetles (Lucanidae) there seems to be a gradient of growth intensity with its centre in the mandibles, falling away posteriorly.[93] Obviously, where growth rates in the body are regulated by such complex laws they cannot be expected to conform to Przibram's simple rule.

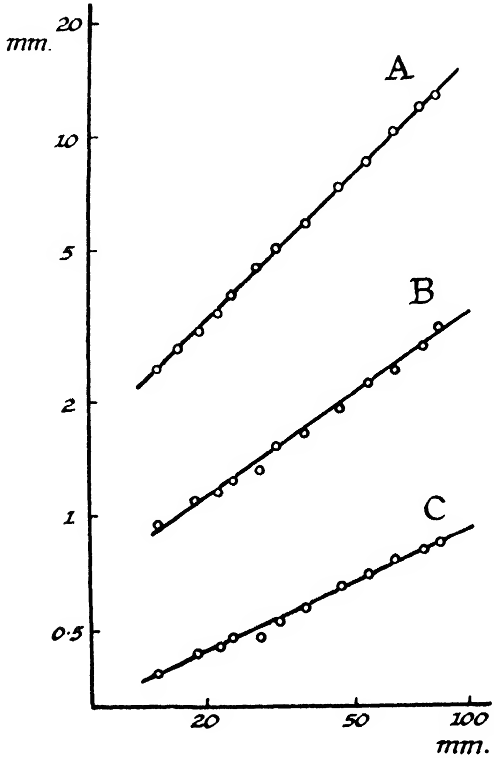

Fig. 28.—Heterogonic growth in *Dixippus morosus*. Logarithmic co-ordinates

Length of posterior segment of prothorax (*A*), width of head (*B*), and diameter of eye (*C*) plotted against the total length of the body (*after* Teissier).

Number of instars—Simple rules which seek to relate amount of growth with moulting break down also where the number of instars is inconstant. Such inconstancy is very common.[91, 97, 194] Variations from 10–14 occur in the small wax moth *Achroea grisella*,[26] from 5–27 in the May-fly *Baëtis*. Often the female tends to have more moults than the male, as in *Sphodromantis*,[154] *Dermestes*,[112] *Tineola* and many other insects.[194] Where extra instars occur, the condition may be hereditary; different races of *Bombyx mori* have the number of ecdyses determined by mendelian factors [136]; and the occurrence of an extra instar in females of *Locusta* may be inherited.[98] Or the number may be determined by external conditions; raised temperature increases the number of moults from 9–11 in *Sphodromantis*,[154] from 4–5 and 5–6 in males and females respectively of *Dermestes*,[112] from an average of 4 at 18° C. to an average of 5 at 25° C. in *Ephestia kühniella* [68]; it has the reverse effect in *Melanoplus mexicanus* and in *Pieris brassicae* [91]: the larva of *Pieris* has 5 moults at 14–15° C., 4 at 15–20° C., 3 at 22–27° C.[101]

Inadequate nutrition, causing a prolonged larval period, may increase the number of moults enormously, especially in insects living in dry foodstuffs: by keeping the clothes moth *Tineola* on a rich or a poor medium the larval period could be varied from 26 days with 4 moults to 900 days with 40 moults.[194] In such cases moulting can take place without growth; indeed the insect may grow gradually smaller. In most insects moulting ceases when the body is fully grown; but in Apterygota moulting may continue in the adult without change of size or organization.[155, 185]

Causation of moulting—There are two aspects to the problem of moult-

ing: (i) the initiation of the process of growth and cuticle formation, and (ii) the determination of those changes in form that occur during ecdysis. Changes in form we shall consider later; here we are concerned only with the first aspect. And from this point of view there is no need to separate moulting from pupation; the essential phenomena of growth and cuticle formation are common to both.

Moulting involves all parts of the body, whether supplied with nerves or not, at the same time. If spines or appendages are transplanted from one caterpillar of *Vanessa* to another, they moult synchronously with their new host; limbs of *V. io* and *V. urticae* may be interchanged; or limbs of a 4th instar may be implanted on a 3rd; and always the time of moulting and the number of moults is determined by the host.[18] The same is found with transplants in *Dixippus* [126] and *Galleria* [143]; and internal transplants (ovaries) in *Celerio* develop synchronously with their host.[36] These experiments suggest that moulting is determined by factors outside the epidermis, perhaps hormones circulating in the blood.

Moulting hormone—A hormone is certainly responsible for causing moulting in each instar of the blood-sucking bug *Rhodnius*.[203] In each nymphal stage this insect takes only one gigantic meal of blood, and moulting occurs at a definite interval thereafter; an interval of about 12–15 days in the first 4 stages, about 28 days in the 5th or final stage. During the first few days after each feed removal of the head effectively prevents moulting, although the headless insects may sometimes remain alive for longer than a year. There is, however, a 'critical period', about 4 days after feeding in the first 4 nymphal stages, about 7 days after feeding in the 5th stage, after which moulting is not prevented by decapitation (Fig. 29). The blood of an insect which has passed

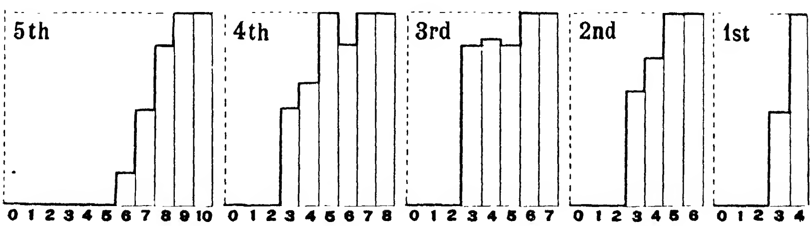

FIG. 29.—Charts showing the proportion of *Rhodnius* nymphs (1st to 5th instars) which moulted, out of batches decapitated at different times after feeding

Ordinates: percentage moulting. Abscissae: days after feeding (*after* WIGGLESWORTH).

the critical period will induce moulting in an insect decapitated immediately after feeding—as can be shown by decapitating the two insects and connecting them together by a small capillary tube sealed into the neck of each with paraffin (Fig. 30). The 'moulting hormone' which the blood evidently contains will cause moulting in any stage: the blood of 4th-stage nymphs will cause 1st-stage nymphs or 5th-stage nymphs to moult; it will cause moulting in newly moulted unfed nymphs; and it will induce moulting in *Triatoma*, an allied genus, and in *Cimex*, which belongs to another family (Cimicidae). Clearly it is not peculiar to the species.

Similar evidence of the secretion of a hormone has been obtained for the pupation of Lepidoptera. In *Lymantria* there is a 'critical period' between

the seventh and tenth days after the last larval moult. If the caterpillar is ligated and cut in two at 7 days, the anterior half pupates some 8 days later, the posterior half shows no change, though it survives for many weeks; while if ligatured and cut at 10 days, both parts pupate.[107] In *Galleria* larvae, which stop feeding 4–5 days after the last larval moult, and pupate at about 9 days, the critical period occurs at about 8 days; in *Bombyx mori*, which stops feeding at 12–13 days and pupates at 18–20 days, it occurs at about 9 days, that is, while the larvae are still feeding.[28] In caterpillars of *Ephestia*, ligation during the critical period may result in pupation in the posterior part of the larva being limited to flecks or patches; perhaps these are the most 'sensitive' regions of the epidermis.[116]

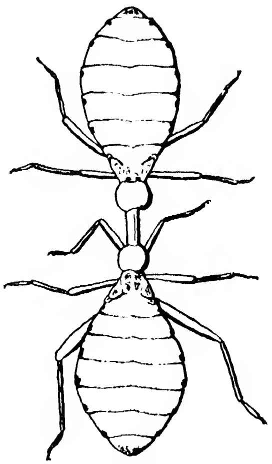

FIG. 30.—Two 4th instar nymphs of *Rhodnius* decapitated and connected by a capillary tube sealed into the neck with paraffin wax (*after* WIGGLESWORTH)

Likewise in the larva of the blow-fly *Calliphora*, if this is ligated less than 12 hours before it would normally pupate, formation of the puparium occurs on both sides of the ligature; if ligated more than 12 hours before, puparium formation is limited to the anterior half (Fig. 31). Injection of blood from a pupating larva into the posterior fragment of a non-pupating larva will cause this to pupate[63]; and pupation may be induced also, by the injection of suitably prepared extracts, not only from other fly pupae, but from young pupae of the wax-moth *Galleria*.[144a]

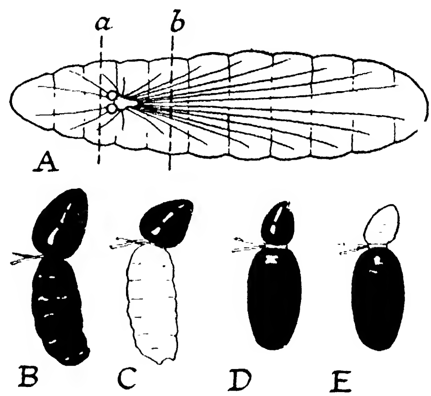

FIG. 31.—Muscid larva, showing the ganglion and the nerves, and the positions *a*, and *b*, at which ligatures are applied

B, larva ligatured at level *b* after the critical period: posterior fragment paralysed; both fragments have pupated. *C*, larva ligatured at same level before the critical period; pupation only of the anterior fragment containing the ganglion. *D*, larva ligatured at level *a*, after the critical period; both parts pupated; posterior part not paralysed. *E*, larva ligatured at same level before the critical period; pupation only in posterior fragment (*after* FRAENKEL).

Source of the moulting hormone—Many organs and tissues have been suggested as possible sources for the hormone which induces moulting or pupation.

(i) *The oenocytes* [105]—These certainly show a cycle of secretory activity with a peak in the middle of each moulting stage (p. 243). But it seems more likely that they play a part in the intermediary metabolism of moulting, than that they secrete the hormone; because (in *Rhodnius* at least) they do not reach the height of their activity until the new cuticle is being laid down, and this occurs long after moulting, as indicated by the multiplication of the epidermal cells, has begun.[203] On the other hand, there is an abnormal strain of silkworms which fail to moult; and when these are compared histologically with a normal moulting strain,

the first difference to appear is in the oenocytes; in the normal insects these become enlarged.[205]

(ii) *Verson's glands* [34, 90]—These, as we have seen, pour out their secretion by a duct beneath the old cuticle,[173] and there seems no reason to doubt that they contribute to the moulting fluid (p. 25). They show their maximum activity in the final stages of moulting, and in *Rhodnius* the corresponding glands are scarcely differentiated by the critical period when moulting definitely begins.[203]

(iii) *The corpus allatum*—The corpora allata are small glandular organs, nests of cells which in the course of embryonic development have been budded off from the ectoderm between the mandibular and maxillary segments. They are innervated from the brain by way of the posterior sympathetic ganglia, in close relation to which they usually lie. Formerly believed to be nerve ganglia, they are now generally regarded as organs of internal secretion [94, 133] (p. 105).

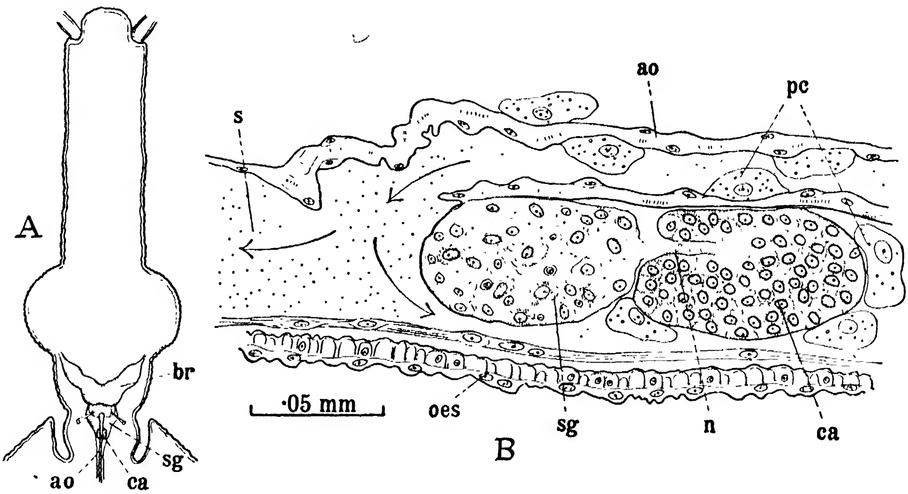

FIG. 32

A, head of *Rhodnius* from above to show position of corpus allatum; B, longitudinal section of corpus allatum and related structures (*after* WIGGLESWORTH). *ao*, aorta; *br*, brain; *ca*, corpus allatum; *n*, nerve to corpus allatum; *oes*, oesophagus; *pc*, pericardial cells; *sg*, sympathetic ganglion.

In *Rhodnius* the cells of the corpus allatum (as in some other Hemiptera the two glands fuse to form one) (Fig. 32) become conspicuously swollen during the critical period, when the hormone is being secreted,[203] and the blood of nymphs which have passed the critical period seems to be a better source of the moulting hormone when they are allowed to retain the corpus allatum than when it is removed with the head.[203] There is, however, no conclusive evidence that this gland secretes the hormone. There is no difference between the corpora allata in the normal and the non-moulting strain of silkworm.[205] Removal of the corpora allata in Lepidoptera (*Bombyx mori*) [28] before the critical period does not prevent pupation; and Orthoptera (*Melanoplus*,[199] *Dixippus* [142]) will still moult after they have been excised.

On the other hand, in the flies *Calliphora* and *Drosophila*, in which the 'ring gland' or Weismann's ring surrounding the aorta just above the cerebral lobes is the homologue of the corpora allata (Fig. 33),[35] there seems no doubt

that this gland is the source of the hormone which induces formation of the puparium. For in *Calliphora* the gland becomes vacuolated during the critical period,[35] and in *Drosophila*, if it is transplanted from mature larvae into young larvae or into the posterior halves of larvae ligated before the critical period, these are caused to pupate (*cf.* p. 56).[81]

(iv) *The brain*—Caterpillars will still pupate if the nerve cord is cut behind the head before the critical period; but if the brain is removed at this time (in *Lymantria* [107] and in other Lepidoptera [28, 116]) pupation is prevented; and in a few instances (in *Ephestia*,[116] *Sphinx*,[145] and *Deilephila* [145]) the subsequent implantation of the brain, without the corpora allata, into the abdomen of such larvae has induced pupation. These experiments suggest that in Lepidoptera the brain itself may be the source of the secretion.

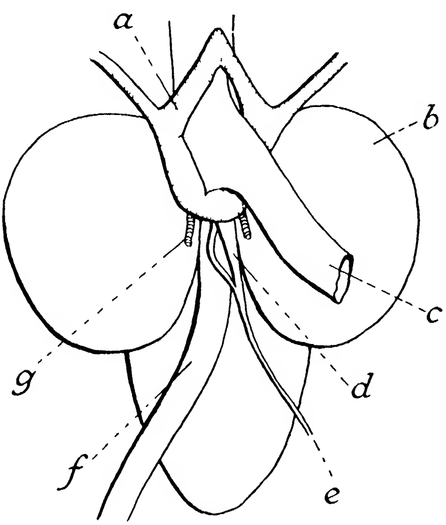

FIG. 33.—Weismann's ring (the homologue of the corpora allata) in *Calliphora* (*after* BURTT)

a, Weismann's ring; b, brain; c, aorta; d, branch from recurrent nerve to Weismann's ring; e, recurrent nerve; f, gut; g, trachea to Weismann's ring.

(v) *The epidermis*—The fact that decapitation or removal of the brain prevents moulting suggests that the secretion which initiates growth is produced in the head. But it is possible that once the process has been started, chemical factors from the growing cells themselves ensure its continuation.[203] Injuries to the epidermis produce localized growth with the secretion of new cuticle as in moulting (p. 63).[204] Perhaps in some insects the power to initiate growth resides in the general epidermis and no exogenous source of hormone exists.

Stimulus to secretion of moulting hormone—Different insects are certainly caused to moult by different stimuli. The blood-sucking bugs *Cimex* [97] and *Rhodnius* [203] will not moult if they are starved; and if given small meals they will live indefinitely without moulting. In them it is not the state of nutrition but the distension of the abdomen by the meal which provides the stimulus to moulting. This stimulus is probably carried to the brain by the nerves, for section of the nerve cord prevents secretion of the moulting hormone.[203] Moulting in these insects cannot be regarded as a form of excretion (p. 28). On the other hand, absolute starvation will usually precipitate pupation in caterpillars (pupae of *Galleria* one-tenth the normal weight have been produced [130]) while underfeeding or intermittent starvation cause pupation to be deferred.[107, 140, 149] The same is true of mosquito larvae.[202] Larvae of *Tenebrio* and some other insects will moult several times although starved; starvation seems even to stimulate the process sometimes, as in the clothes moth *Tineola*, which may develop a sort of 'moulting frenzy' and cast the skin as many as eight times in two or three days without change of size.[194]

Regeneration of appendages tends to increase the number of moults in *Sphodromantis* (see p. 65) [154] and to cause moulting in the adults of *Machilis* (Thysanura).[155] This suggests a relation between the factors responsible for wound healing and for moulting. The healing of wounds (a form of localized moulting) can take place in the adult (p. 63). The absence of moulting in most adult insects is probably due to failure of the moulting hormone; for imaginal organs of the earwig *Anisolabis* transplanted to the nymph will grow on and will moult when their host moults [66]; and adult bed-bugs *Cimex* may be caused to moult by transfusing them with blood from moulting nymphs of *Rhodnius*,[204] as also can the adults of *Rhodnius* itself.

METAMORPHOSIS

Definition of metamorphosis—While growth in size is one purpose of moulting, change of form is another. Since the outward form is determined by the cuticle and can change only when the cuticle is shed, change of form is even more strikingly discontinuous than change in size. The intermediate stages of development become apparent only under special circumstances. There are all degrees of such discontinuity. In the Apterygota the gradations are extremely slight; the insect becomes sexually mature at some indefinite point, and even then moulting may continue.[155] Such insects are termed Ametabola: attaining the adult stage without a metamorphosis. In all other insects, although the change of form is generally slight at the earlier moults, there are conspicuous changes in the final stages. The adult stage is generally winged; its sexual appendages suddenly become prominent; and if its mode of life is changed it may suffer a dramatic transformation.

From the standpoint of phylogenetic classification, great importance is attached to whether the wing buds in the early stages are visible externally (Exopterygota) or concealed beneath the cuticle until metamorphosis begins (Endopterygota); and whether the active immature nymph * is transformed directly into the adult (Hemimetabola, showing 'incomplete metamorphosis') or whether a pupal stage, incapable of feeding and often comparatively inactive, is interposed between the larva and the adult so that metamorphosis takes place in two stages (Holometabola, showing 'complete metamorphosis'). From the standpoint of physiology, although it is convenient to use these terms, such distinctions are untenable. The histological processes of development are of the same type in all, though there are differences in degree. We shall therefore follow Handlirsch [82] in applying the term 'metamorphosis' to the more or less marked change of form which occurs when the insect becomes adult; whether this change requires two moults and a pupal stage or a single final moult.

Histological changes—During moulting, even when unaccompanied by any striking change of form, there is much reconstruction in the epidermis. In *Galerucella* (Col.),[147] *Hyponomeuta* (Lep.) [92] and aquatic Hemiptera [146] numerous cell divisions take place, and before or during their occurrence little spheres of chromatin appear in the cells and are set free and engulfed by phagocytes. These droplets are believed to be discharged from the nuclei;

* The young stages of exopterygote insects are termed 'nymphs' by English authors; but there is much to recommend the German practice of calling these stages 'larvae'.

they have been thought to constitute a visible sign of a process of ' rejuvenation ' or ' dedifferentiation ' which permits the cells to renew their growth. But many of these chromatic spheres doubtless come from the dissolution of entire nuclei, as in *Calandra* (Col.) (Fig. 34).[132] Perhaps the majority arise in this way ; for if the epidermis of *Rhodnius* is examined in surface view at this stage, a great part of the epidermal cells, oenocytes and dermal glands are seen to be undergoing cytolysis. In fact, alongside the ' histogenesis ' of the new epidermis there is a widespread ' histolysis ' of the old.

At the pupal moult of holometabolic insects (Lepidoptera,[92] Coleoptera [147]) precisely the same process takes place over a great part of the body surface. But in those parts of the body and in those insects where the more extreme type of metamorphosis occurs, the rudiments or ' Anlagen ' of the adult organs are already present in the early stages as clusters of undifferentiated embryonic cells, the ' imaginal discs ', the significance of which was first appreciated by Weismann. These arise as thickenings of the epidermis, often with loose accumulations of mesenchyme cells below them. In Diptera they are already evident in the late embryo ; in *Vanessa* (Lep.) those of the wings are present in the first larval stage, those for the limbs appear at the end of the 4th stage (Fig. 35).[19] When metamorphosis occurs, these embryonic germs grow and unfold ; the more specialized parts of the larva at the same time degenerate and disappear.

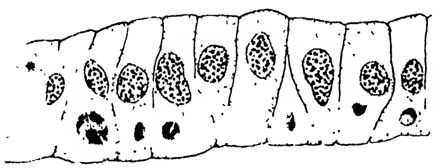

FIG. 34.—Epidermis of young pupa of *Calandra oryzae* showing appearance of chromatic globules (*after* MURRAY and TIEGS)

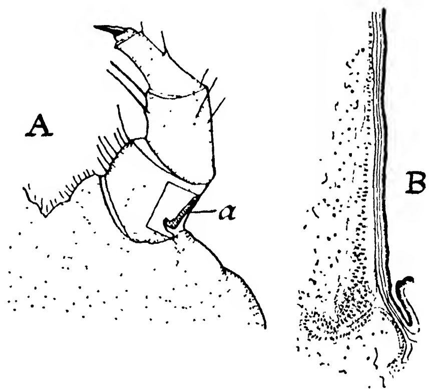

FIG. 35

A, position of the imaginal disc (a) in the leg of larva of *Vanessa urticae* ; B, detail of the imaginal disc. (*After* BODENSTEIN.)

But this process differs from the metamorphosis of hemimetabolic insects only in degree. Even in the extreme metamorphosis of Diptera or Hymenoptera many of the larval organs and cells do not succumb but are reconstituted to form the adult body. There is never demolition and then reconstruction, but progressive substitution. The most specialized larval structures disappear, the most specialized parts of the imago are built anew from the imaginal discs ; but many organs, such as the Malpighian tubes of Diptera and Coleoptera, though more or less reconstructed, are common to both larva and imago. Among the muscles of Muscidae and Hymenoptera there are all grades between survival and replacement.[141]

Histolysis—About the mechanism of histolysis there has been much controversy ; particularly as to the part played by the phagocytes in the blood. At the present time it is agreed that there are great differences in different insects or in different organs of the same insect ; and it is agreed that the

phagocytes are concerned only in removing cells which are already dead and in process of autolysis—although such cells may show little histological change.[4, 141, 193] In the blow-fly *Calliphora*, haemocytes stuffed with granules of disintegrating tissue, the 'Körnchenkugeln' of Weismann, are abundant in the blood of the pupa (Fig. 36).[111, 141, 157] Whereas in the allied blow-fly *Lucilia*, autolysis and fragmentation of the muscles occur spontaneously; the haemocytes appear late on the scene and are concerned only in the ultimate digestion of the muscle fragments.[60] A like diversity is found in Hymenoptera: in the ant *Formica*, salivary glands, Malpighian tubes, fat body cells and many muscles are phagocytosed [141]; in the Chalcid *Nasonia*, many of the tissues dissolve without the intervention of haemocytes, but these fall upon any undissolved fragments present in the blood, and some tissues are attacked when they show little visible change; here chemical autolysis and phagocytosis take place side by side [193]; while in the honey bee there is no evidence of phagocytosis in any organ, whether muscle, fat body, silk glands or gut

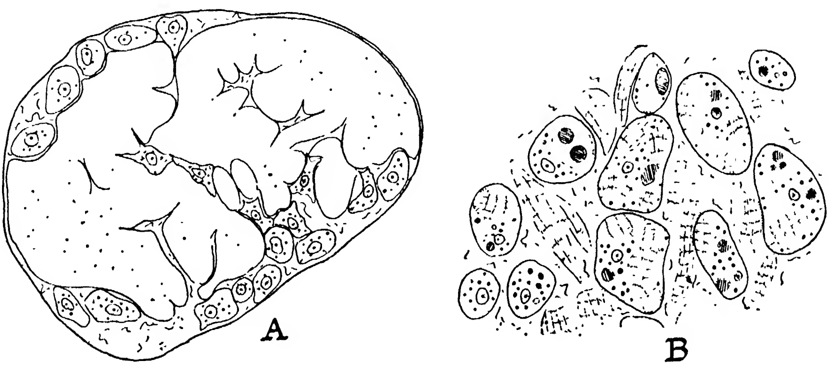

FIG. 36

A, transverse section of larval muscle of *Calliphora* 7 hours after pupation, showing the penetration of haemocytes beneath the sarcolemma and deep into the muscle; *B*, advanced stage in dissolution of the muscle (10 hours after pupation). Haemocytes stuffed with débris to form 'granular spheres'; some muscle fragments (sarcolytes) are still free. (*After* PÉREZ.)

wall.[135, 189] In Lepidoptera, extensive phagocytosis of dead cells may occur in the nerve ganglia [170] and muscles.[13] In *Calandra* (Col.) some tissues are phagocytosed, others not [132]; in *Galerucella* (Col.) phagocytosis is general, many of the cells attacked showing no visible degeneration.[147]

Metamorphosis and embryonic development—Although the mechanisms of histolysis and histogenesis vary greatly in detail in different insects, they always serve a common purpose: the replacement of the larval organism by the adult. The insect may be looked upon as made up of two organisms, larva and imago, existing in a single individual. As we saw in discussing early embryonic development (p. 5), organization is not merely a co-operation among cells; the 'organism' appears to be a differentiated continuum whose division into cells is a late occurrence in development. The 'individuation field' which moulds the body form is something superior to the cells.

This idea of a dual determination in a single body is supported by those experiments on *Drosophila* (p. 8) which show a wave of imaginal determination spreading throughout the egg, quite separate in point of time from the larval determination, and occurring at a stage of development when histological

differentiation of both larval and imaginal structures is entirely wanting. One might therefore regard metamorphosis as the progression of this imaginal determination to the stage of visible differentiation—thereby superseding the larval differentiation which preceded it. This conception would make metamorphosis, what many have considered it, a return to embryonic development.

Control of metamorphosis—Many factors have been suggested as regulating this process, by inhibiting the development of the imaginal discs in the young stages, or by causing the purely larval tissues to disintegrate at metamorphosis. It has been suggested, for example, (i) that the imaginal discs are held quiescent by the excretory products of the active larval tissues, and when these begin to degenerate the adult tissues can begin to grow[5, 163]; (ii) that the larval tissues give out substances which keep the phagocytic blood cells at bay; when they age they become a prey to phagocytes and the imaginal discs develop in their place[111, 157]; (iii) that asphyxia of the larval tissues causes their degeneration[9]; (iv) that the oxidase associated with melanin formation, which is at a maximum at the time of pupation, is in fact the *cause* of metamorphosis[51]; (v) that the increasing size of the larval tissue cells (in the Chalcid *Nasonia*[193] and the beetle *Calandra*[132]) sets a physical limit to their ability to gain nourishment by diffusion; when that limit is exceeded they die; (vi) that secretions from the growing sex glands activate the imaginal discs[141]; (vii) that in the larva, the development of the imaginal discs is checked by secretions from the brain.[107]

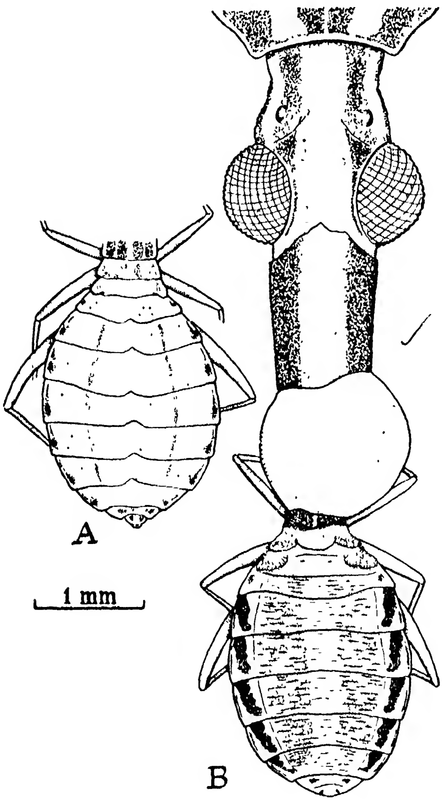

FIG. 37

A, thorax and abdomen of normal 2nd instar nymph of *Rhodnius*; *B*, precocious 'adult' produced from a 1st instar nymph by joining it with paraffin wax to the tip of the head of a moulting 5th instar nymph (*after* WIGGLESWORTH).

These are mostly hypotheses unsupported by experiment; but in recent years evidence has been accumulating that chemical differences do exist in the blood at metamorphosis, and that these are responsible for the morphological changes. Thus the imaginal leg discs of mature blow-fly larvae, grown in tissue culture outside the body, show no differentiated growth in larval blood, whereas in pupal blood they will unfold and differentiate.[64] Limbs of young *Vanessa* (Lep.) larvae transplanted to larvae of a later instar, are sloughed off when their host pupates.[18] Pieces of the integument from larvae of *Galleria* (Lep.) implanted into pupating larvae of the same species, or another species such as *Achroea*, moult to form pupal cuticula synchronously with their new host; further development of these implants to form imaginal

cuticula takes place when the host pupa completes its metamorphosis.[143] And if the young nymphs of *Rhodnius* are transfused with the blood of 5th-stage nymphs, which are in process of moulting to become adult, they undergo a precocious metamorphosis. Even 1st-stage nymphs recently emerged from the egg, if treated in this way, will develop the cuticle and pigmentation, rudimentary wings, abdominal structure and genitalia characteristic of the adult (Fig. 37).[203]

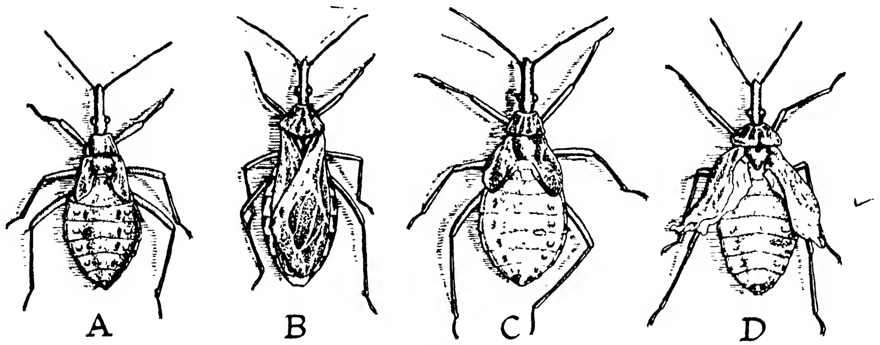

FIG. 38

A, normal 5th-stage nymph of *Rhodnius* ; B, normal adult *Rhodnius* ; C, ' 6th-stage ' nymph produced by implanting the corpus allatum from 4th-stage nymph into abdomen of 5th-stage ; D, ' 6th stage ' similarly produced, showing nymphal cuticle all over abdomen ; wings, thorax, &c., intermediate between nymph and adult. (*From photographs by* WIGGLESWORTH.)

Corpus allatum and control of metamorphosis—The insect seems to be capable of developing its imaginal characters, that is, of undergoing meta-

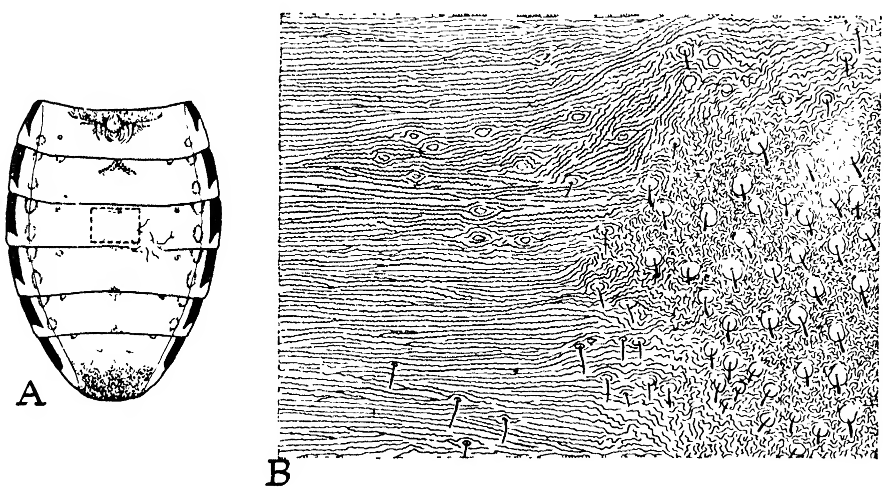

FIG. 39

A, dorsal view of abdomen of *Rhodnius* adult showing area of nymphal cuticle around site of implantation of corpus allatum of 4th instar nymph ; B, detail from A showing nymphal cuticle on right merging into adult cuticle on left. Note vestiges of sockets without bristles in the intermediate zone (*after* WIGGLESWORTH).

morphosis, at any stage, but to be restrained or inhibited from doing so by hormones circulating in the blood. The existence of this inhibitory chemical factor can be demonstrated in 4th-stage nymphs of *Rhodnius* by transfusing their blood into 5th-stage nymphs. These are caused to moult, but they

tion of the prothetely and metathetely produced experimentally in *Rhodnius* (p. 45); the former by providing young nymphs with the blood of 5th-stage nymphs, the latter by implanting into 5th-stage nymphs the corpus allatum of some younger stage.[203]

Prothetely in larvae of *Lymantria* is regarded by Goldschmidt [72] as an example of those developmental anomalies explicable by disturbances in the *rates* of various developmental processes; in this case an upset in the time relations of metamorphosis and evagination of imaginal discs. This idea may be extended to explain the normal restraint of metamorphosis in the young insect, if it be supposed that two processes are set in motion at each moult: (i) differentiation of the adult form and (ii) deposition of the new cuticle. If

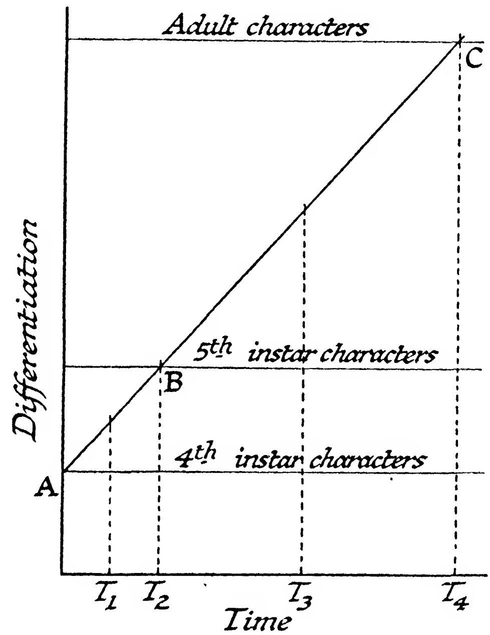

FIG. 42.—Chart to illustrate theory of control of metamorphosis in *Rhodnius*

Ordinates: differentiation of adult characters. Abscissa: time of deposition of new cuticle. In the moulting of a 4th-stage nymph, if the deposition of new cuticle occurs at T_1, characters are intermediate between 4th and 5th instars; at T_2, 5th instar characters appear; at T_3, characters intermediate between 5th instar and adult; at T_4, adult characters.

the second of these processes supervenes immediately after moulting has started, there will be little morphological change; that is, metamorphosis will be inhibited. If the second process is delayed, time will be allowed for the differentiation of adult characters, and metamorphosis will occur. Prothetely or metathetely would represent intermediate stages in a process of which only the end result would ordinarily become apparent (Fig. 42).

This interpretation may be illustrated from the bed-bug *Cimex*. The homologies of the external organs of the male *Cimex* were elucidated by following the development of the epidermal rudiments.[41] If metamorphosis is partially ' inhibited ' in this insect by joining it to a young nymph of *Rhodnius*, it is caused to lay down cuticle before its time, and the genital appendages

appear in an intermediate stage of development, so that the homologies become evident in the cuticular structure (Fig. 43).[203]

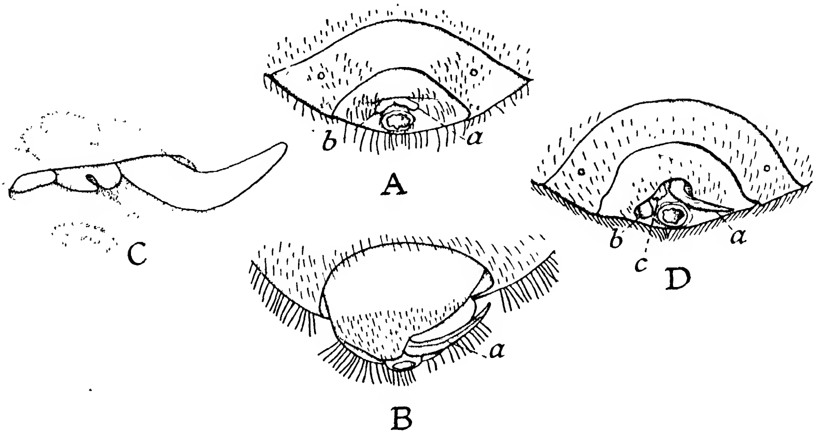

FIG. 43.—Genital segments of male *Cimex*, ventral view

A, normal 5th instar nymph; *B*, normal adult; *C*, epidermal rudiments of the claspers, &c., in a male nymph approaching the adult stage (*after* CHRISTOPHERS and CRAGG); *D*, a stage with characters intermediate between 5th instar and adult produced by transfusing a decapitated 5th instar nymph with blood from a moulting 3rd instar nymph of *Rhodnius* (*after* WIGGLESWORTH). *a*, left clasper; *b*, right clasper; *c*, aedeagus.

Pupal development—In holometabolic insects one instar, the pupa, is normally interposed between the larva and the imago, and the formation of the pupa marks the commencement of metamorphosis. There are, however, departures from this rule. In the higher Diptera metamorphosis begins with the formation of the puparium by the epidermis of the 3rd instar larva; but a delicate cuticle of larval type is moulted within the puparium before the pupa is formed (Fig. 44).[162, 179] In some sawflies there is a 'prepupal' instar, during which no feeding takes place and the salivary glands become modified for spinning the cocoon, before moulting to the pupa occurs.[56] In the males of some Coccids there are two immobile non-feeding stages before the adult is set free. And in some forms, such as Meloidae, there are striking changes in form ('hypermetamorphosis') associated with different modes of life at different epochs of larval development.

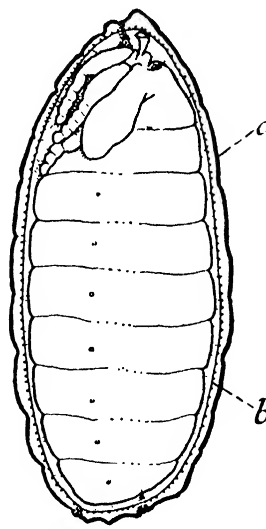

FIG. 44.—
Rhagoletis pomonella

Pupa enclosed in two envelopes, *a*, the puparial shell, *b*, the 'pre-pupal skin'. The pupal head not yet evaginated from the thorax. (*After* SNODGRASS.)

The physiological control of these more complex changes has not been studied; but here we may consider to what extent the continued development of the pupa is dependent on the presence of growth hormones. Conditions seem to vary in different insects. Larvae of *Lymantria* in which the brain is removed after the critical period (p. 37) so that they duly pupate, continue their development up to

the formation of the imago; the brain is not needed to initiate pupal development.[107] On the other hand, if the pupa of *Drosophila* is extracted from the puparium and ligated in the middle between 1–24 hours after pupation, the posterior half does not develop; if the ligature is applied 36–48 hours after pupation, complete development of the posterior half occurs; developmental factors appear to come from the anterior half.[20, 22] Similar experiments on *Vanessa*[80] and *Phryganidia* (Lep.)[22] likewise show that if the abdomen of the pupa is separated from the thorax at an early stage, development of the adult abdomen does not occur. And transplantation experiments show that development of the ovaries in the pupa of *Deilephila* (Lep.) is clearly dependent on hormones in the blood, the source of which is not known.[36] (In *Phryganidia* the ovaries will develop normally even when separated from the thorax; so that they are not dependent on the centre which is necessary for the continued development of the ectoderm.[22])

DETERMINATION OF CHARACTERS DURING POST-EMBRYONIC DEVELOPMENT

Determination of appendages—As in the egg the general pattern of the organism is mapped out or ' determined ' before there is any visible structure (p. 6), so throughout post-embryonic growth the structural details are determined well in advance of their development. The first stage in this

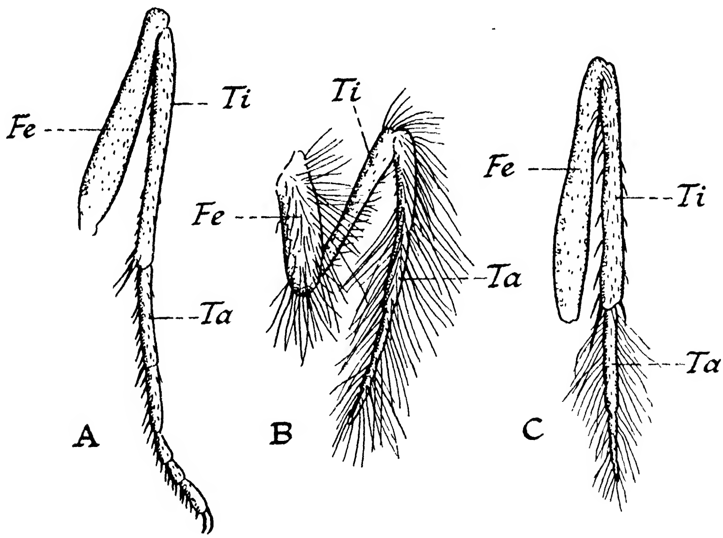

FIG. 45.—*Vanessa urticae*

A, normal hind-leg; *B*, normal fore-leg; *C*, leg produced by implanting larval fore-leg on to stump of hind-leg. The tarsus is chimaerical, combining the scale characters of both fore and hind legs. *Fe*, femur; *Ta*, tarsus; *Ti*, tibia. (*From photograph by* BODENSTEIN.)

process is reached when a given group of tissues is capable of giving rise to an organ with parts duly developed in harmony with one another, although the finer distinctions in the parts are still not fixed. This state is called primary organization (' primäres organsystem ').[19] It is best illustrated from observations on the appendages of *Vanessa* (Lep.).[19] The thickening of the epidermis

which represents the imaginal disc of the limbs does not begin to appear until the end of the 4th larval stage; but already by the 3rd stage the distinctive characters of the adult fore limb and hind limb have been determined. If at this stage a part of the fore limb of the larva is transplanted on to the stump of the hind limb, the two parts work together harmoniously to produce a limb with the normal arrangement of segments (the parts regulate themselves to a state of primary organization), but the characters are chimaerical, combining features of fore and hind limbs (Fig. 45). The same applies to the pupa. The first appendage normally lays down a part of the protective cuticle of the pupal sheath; the third appendage does not contribute to this sheath. This type of cuticle is, however, laid down by a first appendage transplanted to the position of a third in the 3rd larval stage: the character has already been determined though the time when it becomes manifest is dependent on the action of the hormones initiating metamorphosis in the host.

Determination of wing structure and pattern—All through development the characters become determined in an orderly sequence. Each part proceeds from the stage when it is still capable of 'regulation', to the 'mosaic' state (p. 7) long before the growth and movements of the cells put the plan into effect. The process has been followed in most detail in the wings. The system of veins in the wings of the moth *Philosamia* becomes finally determined in the pupa several days old[86]; the venation in *Anax* (Odonata) is partly though not fully determined in the next to last nymphal stage.[137]

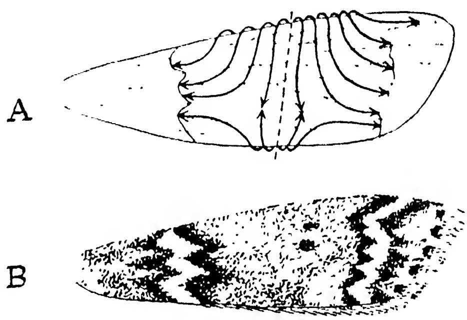

FIG. 46

A, course of spread of the influence determining the symmetrical wing pattern in *Ephestia* (*after* KÜHN); *B*, normal wing pattern of *Ephestia*.

A very clear description of the determination of the wing pattern has been worked out in *Ephestia*.[115] The wing of this moth bears a pattern which is approximately symmetrical about a transverse axis (Fig. 46). On the first day of pupal life no part of the upper surface of the wing is determined in respect to this pattern; by the fourth day determination is complete; and thereafter epithelial defects caused by burning simply obliterate corresponding areas of the mosaic. During these four days the influence (whatever its nature may be), which brings about determination, spreads outwards from the transverse axis of the wing. The course along which the determinative stream advances was worked out by burning small patches of the epithelium; the defects so produced create an obstruction to the spread so that it is possible to tell where it has reached at a given time. Similar results were obtained in *Abraxas grossulariata*.[114] (According to an alternative interpretation of this type of wing pattern, the determinative stream is thought to spread from the wing base in a succession of waves; these give rise to transverse lines, from each of which a second stream then spreads outwards in either direction and so determines the width of the bands to be produced.[118])

The concentric expansion of the pattern zones of the wing, both in this

symmetrical system and in the eye spots, as in *Philosamia*,[86] suggest a parallel with the periodic rings of precipitation (Liesegang rings) which may appear spontaneously in colloidal solutions.[12, 160] But what the nature of the determining influence may be is not known. It does not merely provide different quantities of pigment in the scales of the different zones; it brings about also morphological differences in the wing scales.[113] Partial transection of the wing with a hot lancet early in the pupal stage causes arrested development of scales and sockets distal to the defect.[123] But the two processes are independent, for in *Ephestia* and *Vanessa* the determination of pattern is complete at a time when the form of the scales is still undetermined.[104] In *Philosamia* the details of the colour pattern are determined later than the main outlines of the pattern as a whole, and the factors responsible for this determination, at least so far as they concern scale formation, seem to reside in the blood stream.[86] In the pupal wing of *Ephestia*, mitoses occur most abundantly in those regions which will later be darkly pigmented and along which the wing is folded transversely towards the end of pupal development; and they appear precisely at the time when experiment has shown that determination is complete.[30, 102] There must presumably be some connection between these phenomena.

'Critical periods' in wing development—It has long been known that the wing patterns of butterflies can be altered by exposing the pupae to cold or to heat during a certain critical period. Indeed the seasonal forms of certain butterflies are induced naturally in this way (p. 60). This critical or susceptible period is the time during which determination is taking place; by exposing pupae of *Ephestia* [62] or *Vanessa* [103] to high temperature at different times, it has been shown that there is a succession of such periods during the pupal stage, each relating to a different element in the structure or pattern of the wing. Thus in *Vanessa* pupae exposed for short periods to temperatures of 45–46·5° C., the wing pattern can be modified only during the first 48 hours after pupation; but this period is subdivisible into a great number of susceptible periods for the individual elements of the pattern. From 48–90 hours there is a susceptible period for loss of scales; from 90–102 hours one in which the form of the scales is affected, and so on.[103] In *Pyrrhocoris* (Hem.) the different elements in the wing pattern react differently towards abnormal temperatures.[85]

According to Goldschmidt's hypothesis [72, 75] the colour patterns of the wings of Lepidoptera are caused by different areas of the wing developing at different rates, so that at a given moment only certain areas are prepared to take up from the blood stream those metabolic products which furnish the various pigments. There are thus two processes at work: the development of the wing scales and the production of substances necessary for pigment formation; if the rate of either process is upset—whether by abnormal temperature, by local interference with the blood supply, or by the action of genes—abnormalities in the colour pattern result. But during the whole process of scale formation, the developmental stages run parallel in the scales of all parts of the wing.[104] In *Ephestia* the differences in pattern are associated with differences in the intensity of cell division (see above); there are no differences in the *rate* of development [30]; and if warm or cold tubes are applied to parts of the wing in pupae of *Vanessa*, they will cause local delay or acceleration in development but do not affect the ultimate colour pattern.[69] So that if two competing pro-

cesses are in fact at work they must be processes occurring at an earlier stage than visible differentiation of scale structure.

Action of genes in developmental physiology : rate genes—Most of the characters of the individual are formed in the last analysis by the action of the genes in its chromosomes. In a few cases there is some evidence of the mechanism by which the genes exert their action. One mechanism is that which forms the central point of the theory of heredity elaborated by Goldschmidt [73] according to which the genes influence development by accelerating or retarding definite chains of reaction. There is believed to be a 'genic balance'; the final characters being the resultant of reactions set in motion by genes of different or opposite tendencies. For example, in the wing of *Drosophila* the same alterations of form can be brought about by the action of certain genes or by exposure of the larva to heat during a definite critical period. In either case the result is attributed, in terms of this theory, to a disturbance in the relative velocity of the different processes concerned in determination.[77]

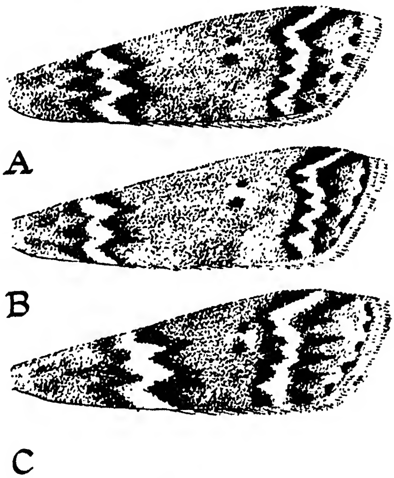

FIG. 47.—Forewings of *Ephestia kühniella*

A, wild type (*sy sy syb syb*). *B*, mutation with widened area between the symmetrical bands (*sy sy Syb Syb*) ; the same appearance can be produced by exposure of pupae of wild type to high temperature from 0–48 hours. *C*, mutation with narrow area between bands (*Sy sy syb syb*) ; the same can be produced by high temperature in pupae 48–72 hours old. (*After* KÜHN.)

Likewise in *Ephestia*, we have seen how the 'influence' which determines the symmetrical pattern of the wing spreads outwards from the axis of symmetry during the four days following pupation. If the pupa is subjected to heat (45° C. for 45 minutes) during the period 12–36 hours after pupation, the symmetrical area in the resultant moth is widened ; a result which corresponds to the action of the mutant gene *Syb* (Fig. 47, B). Whereas pupae exposed to the same treatment 36–72 hours after pupation have the symmetrical area narrowed, as it is by the mutant gene *Sy* (Fig. 47, C). Now we have seen that in the course of determination all the insects pass through a stage with narrow pattern : the gene *Sy* merely has the effect of delaying the determinative stream in its early stages, and arresting it while the pattern is still narrow. Thus the mutant gene seems simply to have influenced in some way the velocity of a developmental process.[115] It is worth noting that the distribution of mitoses in the developing wing of *Ephestia* (p. 52) is shifted by the factor *Sy* and by heat just like the final pigment pattern.[30]

We have seen that prothetely or neoteny in insects is to be explained by some disturbance in the rate of developmental processes (p. 48). In the mealworm this results solely from environmental action and is not hereditary.[119] In *Gryllus campestris* its hereditary character shows that gene action is involved.[46] In *Drosophila melanogaster* there is a so-called 'giant' stock which affords an example of extension of development due to gene action : the larva continues feeding beyond the normal time and perhaps has an extra instar.[54] Similarly,

genes controlling the relative size of parts in accordance with the heterogonic law (p. 35) must presumably act by influencing the *rate* of growth processes.[93]

Autonomous gene action—In general two modes of action by genes are recognized, (i) those whose action is confined to the cells in which they occur: autonomous gene action, and (ii) those which cause certain of the cells to liberate chemical substances and so exert their action at a distance and determine the characters of other tissues.

As an example of the first type may be cited the black-scaled mutant of *Ephestia, bb.* The local action of this gene is well seen in ' mosaics ' in which ' somatic mutation ' (through gene alteration or chromosome loss) has taken place and discrete patches of the recessive black pigment occur in the normal wing (Fig. 48).[113]

In males of *Habrobracon* (Hym.) individuals mosaic for various characters may arise from binucleate eggs, the two oötids having been fertilized by spermatozoa bearing different genes.[79] The mosaic patches may be quite distinct and unaffected by the genes in neighbouring patches. But in males which have eyes mosaic for the two recessive whites (*wh* and ivory), genetically different though phenotypically similar, a line of the normal dark pigmentation may occur along the border of the ivory region. The dominant allelomorph to ' ivory ' present in the ' white ' region apparently causes the production of some substance that diffuses through and reacts with the dominant allelomorph to ' white ', present in the ' ivory ' region.[201] This is an example on the border line between the two types of gene action.

A

B

FIG. 48

A, forewing of black mutant (*bb*) of *Ephestia kühniella*; B, wing of the heterozygous form (*Bb*) with a black patch in the pattern due to somatic mutation. (*After* KÜHN.)

Genes producing diffusible substances—As an example of gene action of the second type may be noted the genes *A*, a^k, *a* in *Ephestia*, which form a graded series in respect to pigment characters; *AA* being black-eyed, *aa* red-eyed.[37a] These genes affect the pigmentation of the larval epidermis, eyes and testis, the pigmentation of the imaginal eyes and brain, the velocity of development and the general ' vitality '. They demonstrate how manifold may be the actions of single genes. Transplantation experiments have shown that the appearance of the various *A* pigment characters is brought about in cells of *aa* constitution by a diffusible substance which is produced in the cells of testis, ovary and brain through the action of the gene *A* and given off into the blood. It is interesting to note that transplantation of an *Aa* ovary is as effective as an *AA* ovary; that is, the production of a definite quantity of ' *A* ' substance is not a direct effect of the *A* gene; production is preceded by a primary reaction in the plasma of the ' hormone '-forming cell. It is in this reaction that *A* is dominant over *a*.[117, 143a] The ' *A* ' substance can also be supplied to *aa* individuals of *Ephestia* by implanting testes from various other Lepidoptera

(*Plodia*, *Galleria*, *Carpocapsa*, &c.) ; a gene with the same properties is common to many species.[143a]

Similar results have been obtained with the eyes of *Drosophila*. Most of the eye colours of *Drosophila* are genetically autonomous ; but there are several exceptions, one of which is vermilion. Vermilion eye colour fails to develop in one eye of a gynandromorph (p. 56) if the other eye is of the wild type [52] ; its colour is influenced by the genetic constitution of the other parts of the body. In such cases if the imaginal discs of the eyes are transplanted from one full-grown larva to another, although they are determined in the embryological sense and develop into eyes, their pigmentation may be influenced by ' diffusible factors ' produced in the tissues of their new host : vermilion eyes assume the wild type pigmentation when transplanted to a wild type host.[58] The v^{+} substance responsible for this effect, which is lacking in the individuals of ' vermilion ' constitution, is liberated from various tissues and circulates in the blood of the pupae between 3–80 hours after pupation ; it is effective if administered to larvae in the food [11] ; but it can be taken up by the eyes only during a restricted period 65–70 hours after pupation. Like the ' *A* ' substance of *Ephestia*, it is non-specific ; it is present in the blood of pupae of *Galleria* and *Calliphora*.[58] It appears, in fact, to be identical chemically with the ' *A* ' substance ; *a* and *v* being, it would seem, homologous genes acting at the same point in the chain of eye colour development.[144]

Effects of the soma of the mother on the offspring—Most genetic characters, as we have seen, are autonomous and not influenced by the constitution of other tissues. Similarly, they are not influenced by the constitution of the soma of the mother. Ovaries transplanted from one strain of *Lymantria* to another produce offspring quite unaffected by the somatic characters of their new host ; there is no ' somatic induction ' [100] ; and the same is true of many characters in *Drosophila*.[42] On the other hand, where genes cause the liberation of diffusible substances, these may influence the characters of the offspring. We saw that voltinism in the eggs of silkworms is determined by the voltinism of the mother (p. 9) ; and *Ephestia* larvae of the constitution *aa* derived from a mother of constitution *Aa* show *A* type pigmentation in their early stages : their characters are pre-determined by the diffusible substance from the mother (Fig. 49). Later this effect wears off and they show *aa* characters.[117] The same effect in the offspring can be procured by implanting into *aa* mothers the testes or brain from *Ephestia* of *A* type or from *Acidalia* (*Ptychopoda*), *Plusia* or other Lepidoptera.[117]

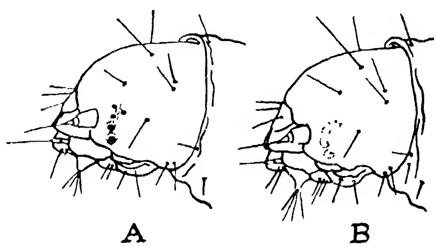

FIG. 49.—Heads of young *Ephestia* larvae both of the genetic constitution *aa*

A, derived from mother of constitution *Aa*, shows *A* type of eye pigmentation ; B, derived from mother of constitution *aa*, shows *a* type of eye colour (*after* KÜHN).

Genes and the production of growth hormones—Genes may be responsible for the production of the growth-promoting hormones, and by causing the failure of these at various periods of growth they can exert a lethal action which may supervene quite late in development. The strain of *Drosophila* known as ' lethal giant ' produces large bloated larvae which fail to form

the puparium; but if the ring gland which secretes the pupation hormone (p. 40) is implanted into them from a normal larva, pupation will take place [81]; the ring gland and its constituent cells being considerably smaller in larvae of the *lgl* strain than in the normal larva.[171] And if the Sphingids *Celerio gallii* ♂ and *C. euphorbiae* ♀ are crossed, the female pupae fail to produce imagines; but the male pupae complete their development. In the female pupae it appears to be the growth hormones which are lacking; for wing germs and ovaries from the hybrid ♀♀ will develop normally if transplanted into the hybrid ♂♂.[36]

Determination of sexual characters—Determination of sex is a special case of the action of genes in development. It is generally agreed that sex is primarily determined by the chromosomes of the fertilized egg. One chromosome, the X-chromosome, of one sex is either unpaired (XO) or paired with a chromosome visibly different from itself (XY). Such an individual is heterogametic, for at meiosis when the chromosomal partners are separated, gametes of two sorts (X and O or X and Y) will be produced. The opposite sex has two X chromosomes and will therefore produce gametes of one sort only. In most insects (*e.g. Drosophila*) the male is the heterogametic sex; the Lepidoptera are exceptional in showing female heterogamety. We shall discuss in a later chapter (p. 404) those variations in the relative numbers of the sexes that are determined by the behaviour of the male and female gametes and their chromosomes during maturation and fertilization. Here we shall consider only the physiological mechanisms by which the sexual characters are controlled in the course of development.

Gynandromorphs—Sex determination is an example of the first type of gene action. With few exceptions, to be noted later, the effect on the development of both primary and secondary sexual characters is exerted apparently within every cell in the body without the intermediation of hormones circulating in the blood. The most conclusive evidence of this is afforded by the occurrence of gynandromorphs or sexual mosaics. These are individuals in which the chromosomal combination in the cells varies in different parts of the body; a state of affairs which may be brought about in several ways. In *Drosophila* they mostly result from the elimination of one of the X chromosomes during early cleavage in a female. Thus a female starts with the constitution XX; but some of its cells become XO and therefore male.[131] In many Lepidoptera they result from the fertilization of binucleate ova, one nucleus having the X, the other the Y chromosome.[43, 120] In certain strains of *Bombyx mori* there is an inherited tendency to produce binucleate ova and this is associated with gynandromorphism.[78, 95] In *Habrobracon* (Hym.) they arise from binucleate eggs of which only one oötid is fertilized *; females kept at a high temperature of 35–37° C. give rise to more binucleate eggs and more gynandromorphs.[79]

The result in all cases is that some of the cleavage nuclei are male and some female. When they reach the cortical zone of the egg they become determined for a given part of the body (p. 7) and patches of one sex or the other are produced, depending on the make-up of the cells from which they happen to be formed. Sometimes the two lateral halves of the body are of opposite sexes; occasionally the partition is transverse; often the male and female characters are scattered over the body as an irregular mosaic (Fig. 50). In *Drosophila*, in

* In Hymenoptera, fertilized eggs give rise to females, unfertilized eggs to males (p. 405).

which the germ plasma arises from a single cell, the gonads are always of the same sex in a given individual [131]; in some insects male and female gonads may be mixed.

In gynandromorphs, as in non-sexual mosaics (p. 54), it is evident that the sexual characters cannot be determined by circulating hormones. There is one case, however, in which diffusible factors seem to be concerned in sex determination. Male mosaics of *Habrobracon* (Hym.) may occasionally have feminized genitalia. This phenomenon is perhaps analogous to the production of dark pigment where two recessive white patches come in contact on the eye (p. 54). Such males may perhaps be mosaics of two types of tissue each recessive for a different sex factor, the dominant allelomorphs of both of which are necessary for femaleness; substances diffusing from one region to the other may interact to produce femaleness.[201]

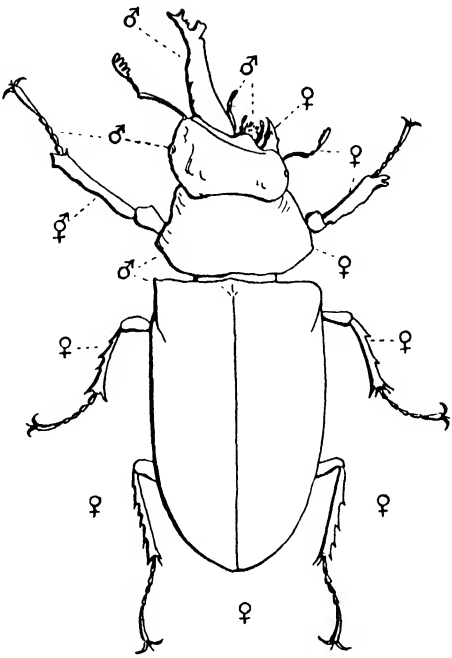

FIG. 50.—Antero-posterior gynandromorph of *Lucanus cervus*

The signs indicate the distribution of male and female characters (*after* V. LENGERKEN).

Secondary sexual characters—At no stage of development do the gonads appear to exert any influence on the sexual characters elsewhere in the body. Castration of *Lymantria* [138] or *Bombyx mori* larvae [96] and of the cricket *Gryllus* [158] has no effect on sexual characters or behaviour. Nor has the implantation of the gonads of the opposite sex into castrated *Lymantria* larvae [129] any effect, even when carried out in very young larvae.[106] The sexual characters of antennae regenerated by such individuals are unaffected by the presence of the implanted gonads [106]; and the characters of wings are unchanged when the germs are transplanted in *Lymantria* larvae from one sex to the other.[106]

Only one exception to such results has been reported. If the wing disc of a female *Orgyia* (Lep.) larva in the 4th instar is transplanted to a male and then extirpated, it regenerates as a male wing; whereas a male wing transplanted to a female regenerates as a male. Unless there is some other explanation for these results, the sex in the former case seems to have been reversed by the host.[139]

Ovaries implanted in a castrated male *Lymantria* may connect up with the cut end of the vas deferens to form a continuous duct [106]; but testes in gynandromorphs of *Drosophila* differentiate only when they are in contact with vasa deferentia; testes attached to oviducts degenerate. Perhaps diffusible substances from the genital ducts interfere with the development of the gonads of the opposite sex.[53]

Genic balance : Intersexes—Although these observations prove that sexual characters in insects are autonomous and not influenced by circulating

hormones, yet it is possible even in insects for partial or complete reversal of sex to occur. The most familiar example of this is to be seen in the intersexual forms of the Gipsy Moth (*Lymantria dispar*) as studied in great detail by Goldschmidt.[74, 75] As in all Lepidoptera, the female is the heterogametic sex (XY) producing gametes of two sorts, X and Y. Yet it is possible to obtain normally functioning males with this genetic constitution (both secondary and primary sexual characters being reversed) which give rise to the two kinds of gametes; or to obtain individuals with the outward appearance of females but with the XX constitution. Between these extremes all intermediate grades of 'intersexuality' occur.

Goldschmidt explains these results by the theory of 'genic balance' already mentioned. Associated with the sex-chromosomes are factors determining sex: male-determining genes in the X-chromosomes, female in the Y. Further, the female-determining factor in the Y-chromosome is believed to transmit its influence to the substance of the gamete before the formation of the polar body, so that both sorts of egg, X and Y, will contain the female-determiner. The male-determining factor being located in the X-chromosome occurs only in the X-bearing gamete. Thus, if the male-determining factor is indicated by the symbol M, and the female factor by F, then the two kinds of gametes produced by the female, X and Y, will have the constitution MF and F, and the zyzotes resulting from their fertilization will be MMF and MF. Now the sexual characters of the individual are supposed to result from the balance between these M and F determiners, each tending to produce different reactions. Normally in the MMF individual the male factors predominate and male characters appear; in the MF individual the female-determining influence is in excess. The 'strength' of the determiners, however, differs in different races of *Lymantria*, and if a 'strong' female is crossed with a 'weak' male, a single F-determiner may outweigh two M determiners so that the genetic male XX (i.e. MMF) will develop all the characters of a female. The intermediate grades of intersexuality are explained by supposing that if in such an MMF individual the F determiner is only slightly in excess, the insect may start developing as a male and then at a given 'turning point' (and the greater the relative 'strength' of the F determiner, the earlier will this happen) it will switch over and complete its development as a female. It is but another example of characters being determined by the relative velocity of two opposing reactions that influence development (p. 48). We saw that in the case of wing patterns these genetical effects could be imitated by exposing the insect to abnormal temperatures, which are supposed to influence the two competing reactions to a different extent. And so it is with sexual characters; intersexes can be produced by exposing to extremes of temperature pure races of *Lymantria* with normally balanced determiners.[75, 110]

Intersexes and abnormal ratios between the numbers of the two sexes may also appear sometimes when two species are hybridized. Thus the cross between the Lepidoptera *Lycia hirtaria* ♂ × *Poecilopsis rachelae* ♀ produces males and intersexes but no females; and the cross *Lycia hirtaria* ♂ × *Nyssia zonaria* ♀ produces all males. As in the *Lymantria* crosses, this is doubtless due to differences in strength or 'valency' of the X chromosome in the two species.[44]

The normal determination of sex in *Drosophila* is explained by Bridges [32]

by a theory somewhat analogous to that of Goldschmidt, according to which there is a balance between female tendency genes and male tendency genes scattered irregularly over all the chromosomes, sex chromosomes and autosomes alike. Intersexes arise from disturbances in this balance resulting, for instance, from the presence of an extra set of autosomes whose effect outweighs that of the sex chromosomes.

These theories of a genic balance controlling sex provide a means of describing satisfactorily most of the observed facts. Argument has turned chiefly upon the nature of the turning point. Goldschmidt supposed that this occurred at a definite time ; organs determined before that time were of one sex, those determined later were of the other. But there are many exceptions to this rule ; intersexes of a given grade do not always show the same distribution of male and female characters ; and intersexes resulting from crossing the parthenogenetic Psychid *Solenobia triquetella* with males of the bisexual race of the same species have their sexual arrangement very patchy and asymmetrical.[174] Therefore some authors favour the view that there is no switch over but that the disturbance of genic balance results in an intermediate type of development throughout the period of growth.[8] Others consider that a switch over does exist, but is not restricted to one point of time ; it may occur at different times in different cells, even in different parts of the gonads.[174]

Parasitic reversal of sex—Another example of sex-reversal is seen in Hymenoptera parasitized by *Stylops*. In *Andrena* invaded by *Stylops* the yellow markings on the face of the male are lessened ; they are caused to appear on the face of the female. As in the intersexes of *Lymantria*, those characters which are differentiated latest in development tend to be most readily affected by the parasite.[167] The effect seems to be related to the abstraction of nutriment from the host ; for several parasites have more effect than one, the large male *Stylops* has more effect than the female, and no effect at all is produced in such hosts as *Polistes* and *Vespa*, which are fed by their parents according to their need, but only in such forms as *Andrena*, *Odynerus* (Fig. 51), *Sphex*, which are provided with a fixed ration by the egg-laying female. The stylopized specimens may be looked upon as intersexes in which the 'turning point' has occurred earlier in the most heavily parasitized individuals. According to this hypothesis, the sex determiner (often thought of as an 'intracellular hormone'), which would normally have the upper hand, is weakened or inhibited by lack of nourishment, so that the latent determiner of the opposite sex is enabled to exert its action and bring about a partial reversal of sex.[167] It is interesting to note that among several Hymenopterous parasites (*Pimpla* [39, 175] ; *Alysia* [89]) males preponderate amongst those emerging from small hosts. But whether their sex is determined by the failure of fertilization in the egg-laying female (p. 405) or by lack of nourishment during growth is uncertain. If several individuals of the egg parasite *Trichogramma* develop in a single host an increased propor-

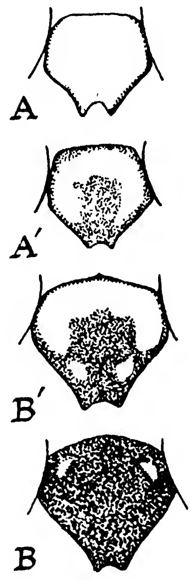

FIG. 51.—*Odynerus perennis*

A, clypeus of normal male ; A′, stylopized male ; B′, stylopized female ; B, normal female (*after* SALT).

tion of males results; but this is attributed to the males competing more successfully under these conditions.[168]

Polymorphism—In many insects which occur naturally in more than one form the characters are controlled by genetic factors; but there are others in which polymorphism may be brought about in the course of development by environmental factors acting upon insects of uniform constitution. Some of these cases may be considered here, since they provide further examples of the mechanism of determination.

The seasonal dimorphism of butterflies seems, in some instances at least, to be an example of external factors influencing the determination of wing pattern. The summer form (*prorsa*) of *Araschnia levana* is modified into the spring form (*levana*) by exposure to cold during a brief susceptible period following pupation; and the degree of modification is proportional to the length of time that elapses before emergence—the slower the individual development, the greater is the modification into *levana*.[183]

When an insect shows well-marked heterogony (allometry) (p. 35), polymorphism may be simply a result of absolute size—variations in the quantity of

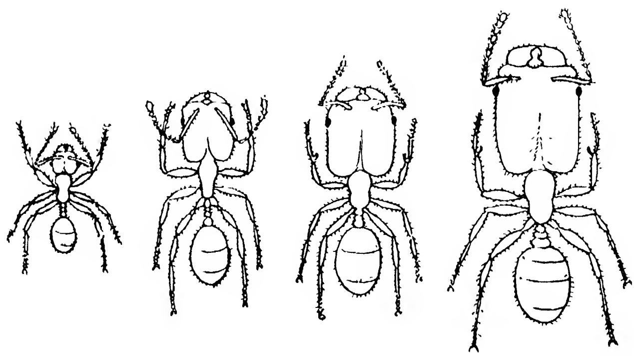

FIG. 52.—Heterogony as a cause of polymorphism. Neuters of *Pheidole instabilis* showing increase in the relative size of the head with absolute size of the body (*after* WHEELER)

food having caused the larvae to pupate early or late in development. This is well seen in the mandibles of Lucanid beetles and in the head types of polymorphic ants; in the large individuals the mandibles and head become disproportionately large (Fig. 52).[93, 200]

Polymorphism in Hymenoptera—In the honey-bee the determination of characters in the females is again effected by nutrition, but here there are qualitative differences in the food. Larvae fed throughout with 'royal jelly' from the salivary glands of the nurses become queens; those fed after the third day with honey and pollen become workers; while intermediates appear if the diet of royal jelly is resumed in the late stages of larval growth.[200] The nature of the determining factor in the royal jelly is not known. Perhaps differences in diet determine also the polymorphism of termites, in which the small-headed workers, the large-headed soldiers with modified mandibles, the 'nasuti' with large head and unpaired frontal process may be either males or females, partly degenerate and wingless.

In ants such as *Pheidole* the distinction between female and worker is thought by some to be predetermined genetically in the egg [197]; but their form is very

labile and susceptible to far-reaching modification by subsequent events. Simple lack of food may affect the proportions of the body by its effects on heterogonic growth. Invasion by parasites, notably the Nematode *Mermis*, may have the same result as malnutrition ; but in addition it seems to have a markedly feminizing action, workers or soldiers assuming the characters of

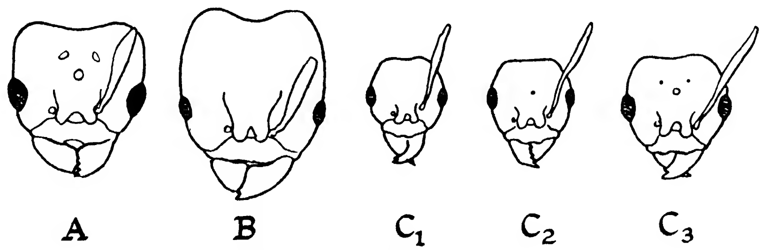

FIG. 53.—*Pheidole commutata*

A, head of queen ; *B*, head of soldier ; C_1, head of normal worker ; C_2 and C_3, heads of workers parasitized by *Mermis* (' mermithergates '). (*After* WHEELER.)

females (' ergatogynes ') (Fig. 53).[200] These ' intercastes ', like the ' intersexes ' produced by stylopization in Hymenoptera, are thought to arise through the parasite changing the relative valency of the hereditary genes and rendering dominant (more or less) those which determine the female caste and which are normally inactive in the neuters.[197]

Effects of quantitative and qualitative differences in diet are seen also in parasitic Hymenoptera. The Ichneumonid *Pezomachus flavicinctus* is large and fully winged when reared from large hosts, tiny and apterous from small hosts [192] ; and males of *Trichogramma semblidis* are apterous when reared from the eggs of *Sialis*, but winged and with striking differences in legs and antennae when reared from eggs of *Ephestia* and other Lepidoptera (Fig. 54).[169] Perhaps the dimorphism in the agamic and gamic generations of female Cynipids (p. 401) is due to their developing in different parts of the host plant.[169]

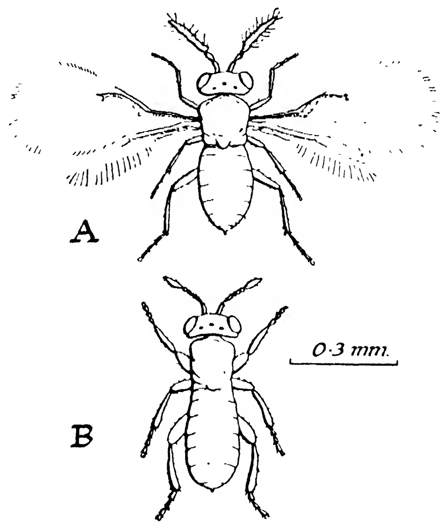

FIG. 54.—Dimorphism in the male of *Trichogramma semblidis* (*after* SALT)

A, winged male reared in eggs of Lepidoptera ; *B*, apterous male reared in eggs of *Sialis*.

Polymorphism in Aphids—Polymorphism is particularly evident among Aphids, in which there are differences on the one hand between the parthenogenic and gamic generations, and on the other between alate and apterous forms among the parthenogenic individuals (Fig. 55). These differences are certainly the result of environmental factors, but the nature of these doubtless varies from one species to another. Most Aphids begin to produce sexual forms in the autumn ; but *Aphis rumicis* has been reared parthenogenetically for nearly 3 years by extending the hours of artificial lighting during the winter months ;

whereas se. ual females were made to appear in June of the first year by limiting the exposure to daylight to 8 hours.[49] And *A. forbesi*, given a $7\frac{1}{2}$-hour day, produced oviparous females in May, while if given a longer day, viviparous reproduction was still occurring in December.[125] This effect of illumination is perhaps really one of nutrition, the plants being affected by the increased light; for the same effect is produced in root-feeding Aphids away from the direct action of the light.[125]

If females of *Macrosiphum solanifolii* which are producing gamic daughters, are transferred from a low to a high temperature, they soon begin to give rise to intermediate forms, and within 10–14 days all the offspring are of the parthenogenic type. The reverse change is brought about by exposing parthenogenic forms to cold. The occurrence of intermediates during the transition periods suggests a switch over from one type to the other in the course of development. But it is interesting to note that during the change from gamic

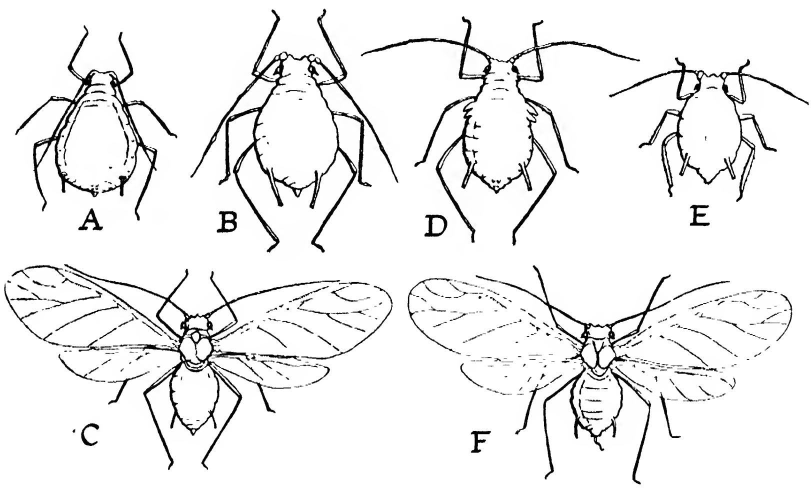

FIG. 55.—Polymorphism in *Aphis malifolii* (*after* BAKER and TURNER)

A, fundatrix; *B*, wingless parthenogenetic female; *C*, winged sexuparous parthenogenetic female; *D*, intermediate form; *E*, amphigonous or sexual female; *F*, male.

to parthenogenic forms the antennae and hind tibiae are first affected in the offspring; whereas during the reverse change from parthenogenic to gamic the body colour and reproductive system are affected first. Such differences in the order of reversal cannot easily be explained on the hypothesis of Goldschmidt that those parts determined latest in development will be influenced first in intermediate forms.[176, 177]

Alate forms can be caused to appear among the apterous viviparous Aphids by various external factors. *Phylloxera* produces winged forms when fed on dry or wilting plants.[176] Lack of water seems to be the primary factor also in *Toxoptera aurantii*; in this insect the critical period during which wing development is most readily affected is at the beginning of larval development.[161] In the Cabbage Aphis, *Brevicoryne brassicae*, the production of winged forms decreases as the protein content in the leaves of the food-plant rises.[61] Inherent factors imposed by the mother may also be involved, alate forms tending to produce apterous offspring; but intermediate forms frequently appear if the

inherent and environmental factors conflict, or if conditions are changed in the course of development (Fig. 55, D).[48, 161] Such intermediates recall the phenomena of prothetely, &c. (p. 47), and they have again been attributed to disturbances in the rates of the developmental processes involved, such as those which fix the time of determination of characters and those which control the secretion of growth-producing hormones.[178] Histological observations on intermediate-winged forms of *Macrosiphum* support, in general, this ' time-of-determination ' theory.[181]

REGENERATION

Healing of wounds—Insects at all ages can repair injuries to their integument. In the early stages of healing the clotting of the blood (in some insects) and the accumulation of blood cells, play a part in the process (p. 236); but

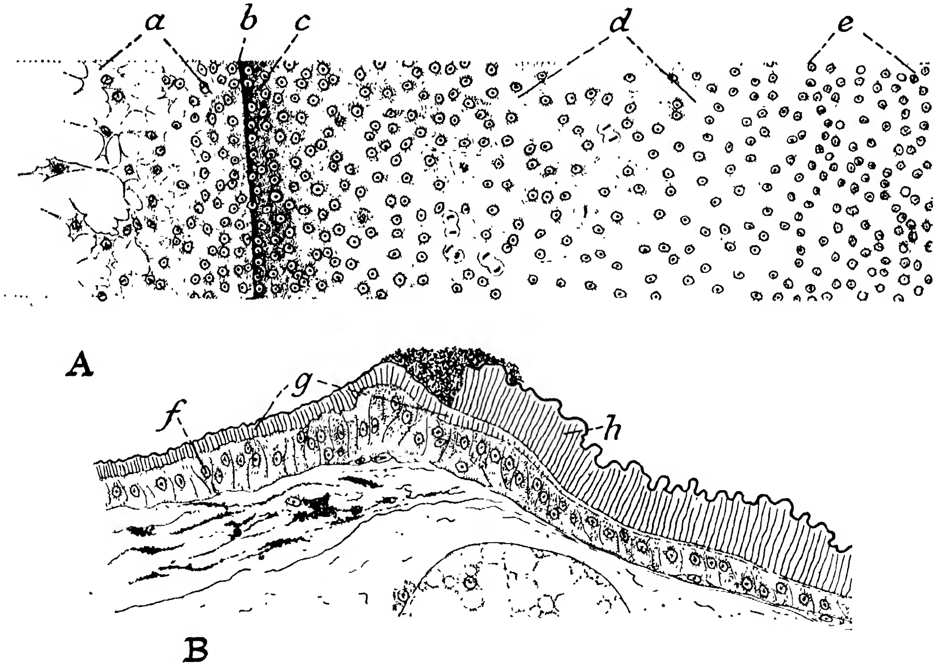

FIG. 56.—Wound healing in *Rhodnius* adult (*after* WIGGLESWORTH)

A, surface view of epidermis four days after a piece of cuticle had been excised. *a*, epidermal cells spreading over the gap ; *b*, margin of excision ; *c*, cells heaped up along margin of wound ; *d*, sparse zone depleted of cells by migration to the wound, cell divisions occurring ; *e*, unchanged epidermal cells. *B*, longitudinal section through the margin of such a wound after three weeks. *f*, epidermis established under excised region ; *g*, new cuticle extending outwards under old cuticle ; *h*, old cuticle at margin of wound.

the essential reactions are those manifested in the epidermis. These reactions have been studied most closely in *Rhodnius*.[204] It appears that the dead or injured cells in the course of their breakdown give rise to substances, products perhaps of the hydrolysis of proteins, which exert an attraction upon the surrounding cells so that these migrate to the wound and congregate thickly around its margin, leaving a peripheral zone where the epidermal cells are very sparse (Fig. 56). If a piece of the integument has been removed, these aggregated cells spread across the wound, make good the defect, and lay down a new

cuticle composed as usual of cuticulin and chitin. Meanwhile cell divisions take place in the sparse peripheral zone and continue until the normal density of the cells has been restored. The process of healing follows the same course in nymphs and adults.

The epidermis will re-establish its continuity when pupae of Saturniid moths, even different species, are cut into segments and joined together.[47] The same happens when the bugs *Rhodnius* and *Cimex* are decapitated and joined [203]; and when appendages are transplanted from one caterpillar to another.[18] In all these cases no other tissues besides the epidermis grow together; but in *Dixippus* the head may be removed and replaced and both integument and gut (though not the nerve cord) are said to grow together again.[124] In many insects, on the other hand, no reunion is obtained on replacement of the head.[14]

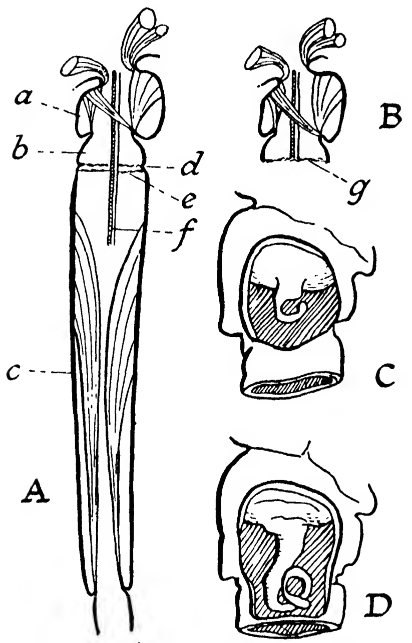

FIG. 57.—Autotomy and regeneration in Phasmids (*after* BORDAGE)

A, upper part of leg showing the junction between trochanter and femur where autotomy occurs; *B*, stump of leg after autotomy, sealed below by the upper half of the haemostatic membrane; *C*, *D*, stages in regeneration of limb within the coxal stump. *a*, coxa; *b*, trochanter; *c*, femur; *d*, groove where the break occurs; *e*, haemostatic membrane; *f*, trachea and nerve; *g*, upper half of haemostatic membrane.

Regeneration—We have seen that during both embryonic (p. 6) and post-embryonic development (p. 50) the parts of the organism, at first indeterminate, become progressively 'determined' or committed to the formation of particular organs. In many cases this determining influence, the so-called 'individuation field' which controls the type of organ that is to be produced, seems to persist in or around the organ long after it has appeared. In such an organ determination is not complete; developmental potencies still remain; and if it is removed it is capable of regeneration.

It has long been known that the appendages of growing insects will reform at later moults if they are removed.[160] Many insects (Phasmids [70] and other Orthoptera,[128] Agrionids,[40] &c.) are capable of detaching an injured limb, usually at the level of the trochanter. This phenomenon of 'autotomy' is particularly well marked in Phasmids. In these the articulation between trochanter and femur has become fixed and no muscles run across the joint (Fig. 57). A two-layered haemostatic membrane crosses the limb at this point; the break occurs between the two layers of this membrane, and the upper portion walls off the little coxal cavity within which regeneration begins. The epidermis first spreads over the terminal scar. It then separates from this and from the cuticular walls of the stump and grows outwards in the form of a papilla which becomes rolled upon itself.[27] But regeneration will also take place after section above or below this level, and in insects not capable of autotomy.[128]

As a rule regeneration can take place only at moulting, and is therefore limited to the young stages; but in Apterygota (*Machilis*, *Lepisma*, *Thermobia*) in which sexual maturity occurs at some indefinite stage, and growth and moulting continue, regeneration can occur in the adult (as it does in Crustacea and Myriapoda)—injury often providing the stimulus to renewed moulting.[155, 185] And in an adult mantis, moulting has been observed after injury, and some regeneration has been noted in the adult in the absence of a moult.[151]

Determination of regenerating organs—The 'individuation field' may extend far beyond the visible organ—as in the imaginal discs of Lepidopterous larvae. If the wing disc of a young larva of *Lymantria* is removed, regeneration and normal wing development takes place unless a considerable area of the surrounding epidermis is excised [20, 195]; under the influence of the individuation field, cells are capable of forming the wing when they would not normally do so. In the same way the cells around the base of the spines of *Vanessa* larvae act as an inductor of the regenerated spine [16]; but this effect is inhibited if the epidermis from another region is implanted on the wound.[17] When an organ, such as a leg, is transplanted to an abnormal site, its individuation field goes with it; and if amputated in its new position it regenerates as a leg.[16] Sometimes it is possible, up to a certain stage of growth, to divide the individuation field: if the imaginal discs of the wings in Lepidoptera are halved in the later larval stages both halves will give rise to a wing.[123]

The capacity for regeneration and the moulding of the outward form seem to reside exclusively in the epidermis. In *Sphodromantis*, antennae and legs of normal form will regenerate after removal of the corresponding ganglia. But they contain no nerve or muscle; only an amorphous granular mass.[184] In *Lymantria*, legs, antennae with normal sensilla, even the epidermal parts of the eye, will develop normally in the pupa after removal of the corresponding ganglia from the larva, though nerves and muscles are wanting.[108] In the course of normal regeneration the new muscles are probably budded off from the epidermal plate which forms over the wound; the nerves by outgrowth from the nerves existing in the stump. The development of the muscles seems dependent on this nerve supply.

Dissociation of larval and imaginal fields—If the limbs of certain beetles, such as *Tenebrio*, are amputated in the young larva, they regenerate in the course of larval life [127]; but in other beetles (*Hydrophilus*, Dytiscids,[127] *Timarcha* [1]) the wound simply heals over and no regeneration takes place until pupation, when a normally organized limb appears. In the latter case the individuation field for the larval organ seems to have been removed with the limb; morphogenetic activity is resumed only when the determination of the adult form is completed at metamorphosis. Amputation must be extended into the thorax in order to prevent this delayed regeneration.[29]

Abnormalities of regeneration: reduplication—The most frequent abnormality in regeneration is for the organ to be duplicated or even triplicated at the tip. When this occurs the arrangement always conforms to Bateson's law: the parts lie in one plane; the inner branch forms a mirror image of the normal; the third or outer forms a mirror image of the inner.[109] In *Mantis* this seems to result from some artificial interference with the wound surface.[152] In Lepidoptera, triplication has been induced by amputating the limb, rotating it 90° or 180° and allowing it to reunite. In these cases, by the use of trans-

planted appendages with recognizable characters, it has been shown that always two branches are derived from the transplant and one from the host (Fig. 58).[21] Duplication may occur in the valves of the external genital organs of certain intersexes of *Lymantria* without preceding injury, and seem to be due to an abnormal degree of separation of parts which ought to be in contact.[71]

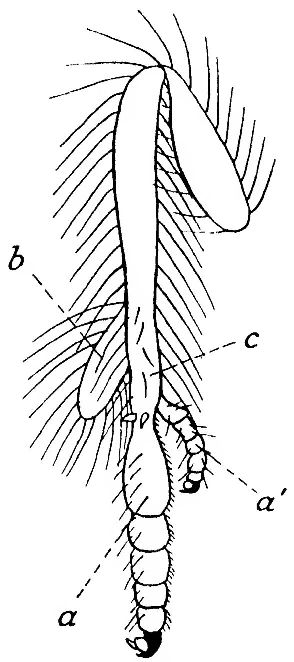

FIG. 58.—Triplicated limb in *Pyrameis cardui* produced by transplanting the two distal segments of the hind-leg (in the last larval stage) on to the fore-leg cut down to the second segment; the transplant being rotated 180°

Two of the branches **a** and **a'** are derived from the transplant; one branch ***b*** is derived by regeneration from the fore-leg. The tibial region ***c*** is chimaerical, combining characters of both limbs. (*After* BODENSTEIN.)

Thus reduplication appears to result from some upset in the spatial relations of the individuation field. In the case of amputated limbs replaced on the stump, if the orientation is normal, the two parts work together to form an organized whole; if inverted they work independently. The basal fragment then regenerates terminal parts; the distal fragment, influenced perhaps by the field or centre in the stump, also regenerates terminal parts.[21]

Homoösis.—Another abnormality is 'heteromorphous' regeneration: the replacement of an appendage by one belonging to another region of the body. In *Dixippus* a fore limb may be regenerated in the place of an amputated antenna (Fig. 59)[31] This is particularly liable to happen if the antenna has been cut through near the base; but there seems to be no causal relation between the removal or retention of Johnston's organ (p. 135) and the type of regeneration,[65] such as has been claimed.[25] In *Cimbex* (Tenthredinidae), if the larva just before pupation receives a slight burn to the antenna, an antenna is developed in the adult; after a severe burn a leg-like appendage arises.[150]

According to Przibram's explanation of this curious anomaly, the regenerating organ contains the potentiality to form both leg and antenna. The reactions which lead to the realization of these alternatives proceed at different velocities, and whichever process is the more rapid becomes dominant. Sometimes the potentiality for forming the normal appendage is temporarily masked: if the antenna in *Mantis* is cut off near the base in the early nymphal stages, a foot-like termination first appears, but is later replaced by a normal antenna.[152] The same idea may be expressed somewhat differently. According to the views of Child, metabolic activity, and with it regenerative potency, shows an axial gradient in the animal body, being most intense at the anterior extremity.[196] If it be supposed that the axial gradient becomes less steep with age, then the potency of differentiation at a given body level may be altered to a potency originally characteristic of a more posterior body level.[93] It must, however, be emphasized once more that the physiological mechanism in the whole process of determination is quite obscure.

However, there does seem to be a certain parallel between heteromorphous regeneration and those developmental anomalies caused by upsets in the rate

of the component processes (p. 48). And just as effects of this type induced by abnormal temperatures can be copied by the action of certain genes, so various examples of ' hereditary homoösis ' are known which simulate heteromorphous regeneration. In *Drosophila*, for instance, the labella of the proboscis

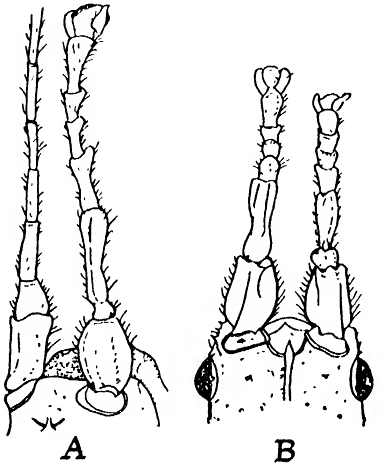

FIG. 59.—*Dixippus morosus*, anterior part of head

A, right antenna regenerated as a leg; *B*, both antennae regenerated as legs. (*From* KORSCHELT *after* CUENOT.)

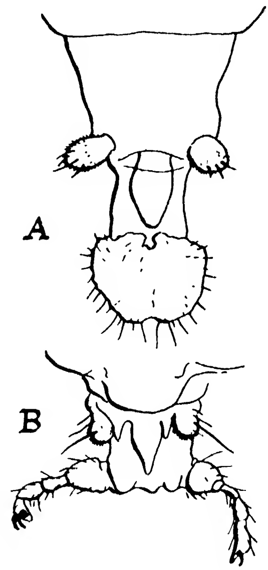

FIG. 60

A, normal mouth parts of *Drosophila*; *B*, mouth parts of *Drosophila* showing proboscipedia. (*After* BRIDGES and DOBZHANSKY.)

may be replaced by legs (' proboscipedia ') (Fig. 60),[33] or the arista of the antenna may develop as a leg (' aristopedia '). The gene responsible for the latter condition is regarded as accelerating leg formation and so enabling the leg differentiation to dominate the antennal.[7]

DIAPAUSE

Types of arrest—We have already discussed the arrest of development in the egg (p. 9). Growth may be arrested similarly in any of the later stages, and, as in the egg, the type of arrest varies in different species and even among individuals of a single species. Broadly speaking, insects fall into two groups: (i) those in which a continuous succession of generations occurs so long as conditions are favourable and in which growth is arrested only by the direct action of adverse circumstances, such as cold, drought or starvation. These are sometimes termed ' homodynamic ' insects.[163] (ii) Those which show at some stage of their life history a prolonged arrest of growth which supervenes irrespective of the environment—' heterodynamic ' insects.[163] Sometimes a heterodynamic generation may alternate with one or more homodynamic generations.

But these two classes represent only the extremes in a graded series. The kind of differences which exist can be best illustrated by examples. The meal-

moth *Ephestia kühniella* will breed all the year round if kept sufficiently warm; but even at the same temperature the duration of development increases during the winter [68]; there is here a hint of a spontaneous arrest related with the season. The blow-fly *Lucilia* will breed continuously if conditions are uniformly favourable; diapause in the larva is brought on by such adverse conditions as poor food, drought, cold,[45] or excessive moisture.[59] But development does not proceed automatically upon return of the larva to a good environment; the arrest may be prolonged for weeks. The hibernation of *Phlebotomus papatasii* (Dipt. Nematocera) is primarily caused by low temperature, but inborn cyclical factors also exist to modify this effect. There is a latent inclination to diapause throughout the year: during the summer there are always some 2–12 per cent. of resting larvae in otherwise active broods; and this number is much increased by exposure to cold; but even when the larvae are kept at 30° C. the proportion which enters this resting state increases during the autumn, and hibernation continues in resting larvae transferred to this temperature.[164, 190] Arrest of growth may likewise supervene in the larva of *Anopheles plumbeus* at any season of the year, though far more frequent in the winter even under the same conditions of temperature and nourishment: the life cycle averages 22–9 days for eggs laid in May or June; 135–271 days for eggs laid in September.[83, 166] In this case the duration of daylight, acting either directly or by its effect on the fauna and flora of the water, may have influenced the results.[6]

The stage at which arrest takes place may be fixed in a species; *Carabus coriaceus* always hibernates as a 1st-instar larva whether it derives from eggs laid in August or in October [57]; many Lepidoptera spend the winter only as full-grown larvae within the cocoon (e.g. *Pionea* and other Pyralids), the majority only as pupae. Or the arrest may occur at varying times: *Melolontha* may hibernate in several larval instars and as an immature adult; *Popillia japonica* (Col.) larvae show a resting period which occurs at different stages of development at different temperatures [121]; in *Reduvius personatus* terms of dormancy may supervene irregularly without any obvious relation to season or to environmental conditions (though they may be brought to an end by heat).[156]

The number of generations which may take place before diapause supervenes, in other words the voltinism, may vary within a single species. This is well seen in the moth *Telea polyphemus*, the voltinism of which varies in different regions of North America. Voltinism in this species is regulated in large measure by environmental factors (exposure of the last larval stage to a falling temperature for about a week induces the pupa to become dormant), but the capacity to respond seems, within certain limits, to be dependent on the genetic constitution of the stock—many stocks being heterozygous in their genetic constitution relating to voltinism.[50] Similar genetic differences have been described in *Deilephila* [84]; and they may be frequent, for it is a common experience during the rearing of a brood of caterpillars in the autumn for a part to pupate and produce another generation and a part to enter a winter diapause.

In general it seems that many of the cyclical diapause phenomena of insects are in fact induced by seasonal changes of one sort or another. But the arrest of development may be determined long before it becomes apparent (we saw that the voltinism of silkworm eggs can be determined in the larval stages of their mother (p. 9)) and so the active factor is liable to be overlooked and a false impression created that there is an internal rhythm. For instance, in

Loxostege sticticalis, in which the duration of the larval phase after the cessation of feeding may vary from 3–180 days, diapause may be induced by unfavourable nutrition for a restricted period in the quite early stages of larval growth; though in this particular case diapause can be prevented at all seasons of the year, whatever the diet, by keeping at a temperature of 32° C.[180]

Physiology of diapause—There are two main hypotheses put forward to explain these phenomena. According to one the arrest is regarded as due to the temporary absence of the factors or hormones necessary to maintain growth.[203] According to the other, growth is thought to be inhibited by some chemical constituent accumulating in the body.[10] Arrest of growth due to lack of hormones is clearly seen in the bug *Rhodnius* (p. 40): the moulting hormone is secreted only after a full meal of blood; if the insect receives too small meals, or if the head is removed soon after a large meal, growth ceases.[203] Perhaps all those insects whose metamorphosis is prevented by removal of the brain may be regarded as being brought to a state of diapause (p. 40).

The arrest of growth by inhibitory substances is an idea developed particularly by Roubaud,[163] who, by analogy with muscular fatigue, regards diapause as a kind of developmental fatigue ('asthenobiosis') which occurs cyclically after a number of active generations and results from an intoxication of the tissues by a surcharge of excretory products which may be transmitted from one generation to the next. It is supposed that this intoxication disappears during a prolonged resting stage at low temperature ('athermobiosis') during which metabolism is much reduced while excretion proceeds. This conception, which has been much elaborated in later publications,[165] is based entirely on hypotheses. The same idea in less specific form has been used to explain the differences in voltinism in different races of a given species by supposing that these are due to the production in different quantities, or at varying rates, of an inhibitory substance ('Latenzstoff'). When this substance reaches a sufficient concentration a latent period or diapause supervenes; in some races this may happen in the first generation, in others in the third.[73] It has further been suggested that the different length of hibernation period in related species may be due to differences in the rate of destruction of 'Latenzstoffe' during the pupal rest, the 'Latenzstoffe' being here identified, as in Roubaud's conception, with metabolic products accumulating early in metamorphosis.[36] And diapause in the egg of *Melanoplus* (p. 9) is pictured as due to a hypothetical 'diapause factor' which increases in amount and inhibits growth, but is gradually destroyed or itself inhibited during exposure to low temperature, thus liberating the inherent developmental factors.[23]

These two hypotheses are not, of course, mutually exclusive: it is possible to imagine the 'diapause factor' as inhibiting the secretion of growth hormones.

Biologically diapause is to be regarded as a means of surviving adverse conditions, notably the winter cold. Exposure to cold is the surest way to 'break' the diapause and bring about renewed growth (*cf.* p. 9). But some insects are exceedingly difficult to arouse from this dormant state: pupae of the moth *Biston* have been known to pass through seven winters before resuming their development.[57] Diapause may also be brought to an end by singeing or pricking the larvae, as in *Lucilia*[163]; by the oviposition of Hymenopterous parasites, as in *Lucilia* attacked by *Alysia*[88]; or by the development of

parasites within the larva, as in *Lipara* (Chloropidae) and *Urophora* (Trypetidae).[198] The mode of action of these stimuli is unknown ; but we have seen that wounds of all kinds provide a diffuse stimulus to growth around them (p. 63) ; it is conceivable that in the cases under discussion this stimulus may lead to the resumption of general growth.[204]

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Chapter IV

Muscular System and Locomotion

ANATOMY AND HISTOLOGY

Anatomy—The muscles of insects fall into two groups; the skeletal muscles forming bands stretched across the articulations of the body wall, which serve to move one segment on another, and the visceral muscles which invest the internal organs. The *skeletal muscles* are made up of elongated contractile fibres lying parallel with one another or converging upon the point of insertion. They are often exceedingly numerous (Lyonnet described 4,061 muscles in the goat-moth caterpillar as compared with 529 in man) and their arrangement differs greatly from one group of insects to another.[53] The *visceral muscles* may form a regular lattice of longitudinal and circular fibres, as around the gut of some insects, the circular fibres being exaggerated at places to form occlusive sphincters; or they may form an irregular feltwork of branching and anastomozing fibres, as in the alary muscles of the heart, in the ventral diaphragm or in the 'peritoneal' covering of the ovaries, where these fibres appear to merge imperceptibly into strands of connective tissue. In some insects, such as the bee, branching and anastomozing fibres of this kind may compose some of the muscle coats of the stomach wall (Fig. 61).[35]

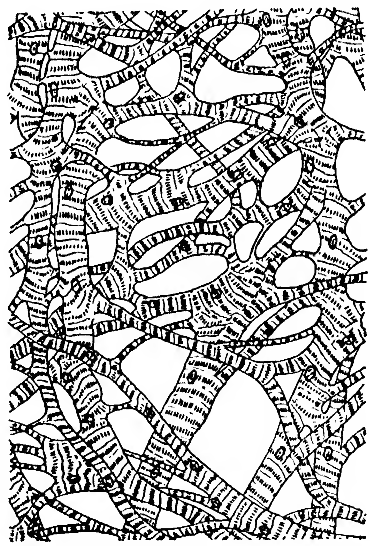

FIG. 61.—Musculature of the crop of *Calliphora*, Dipt., showing the branching and anastomozing fibres (*after* GRAHAM-SMITH)

Histology—All insect muscles seem to be made up of striated fibres; although in some of the visceral muscles the striations may be difficult to detect. Each fibre consists always of a number of parallel fibrillae or sarcostyles laid down in a nucleated plasma or sarcoplasm. But the degree of differentiation of the fibrillae, and their arrangement within the fibre, differs widely in various insects and in the different muscles of the same insect.

(i) In some of the muscles of the honey-bee larva [46] and in the larvae of many Diptera [53] the fibrils are minute threads with little visible differentiation; and they are invested by a thick layer of superficial plasma devoid of fibrillae (Fig. 62, A).

(ii) In most insects the fibrillae are larger; they occupy the whole of the cross section of the fibre and are irregularly arranged. Each fibre is ensheathed

in a relatively tough structureless membrane, the sarcolemma, and the nuclei of the sarcoplasm are either scattered throughout the substance of the fibre or disposed immediately beneath the sarcolemma (Fig. 62, B).

(iii) In the adults of all the higher Hymenoptera and Diptera,[46] and in adult *Dytiscus*,[28] the nuclei are arranged in a row through an axial core of sarcoplasm extending the entire length of the fibre, and the fibrillae, as seen in transverse section, are arranged in flat bundles or lamellae radiating from the centre. These are sometimes called 'tubular muscles' (Fig. 62, C).[35]

(iv) The indirect flight muscles in the thorax of *Apis*, *Vespa*, &c., and many Diptera are sometimes called 'fibrous' or 'fibrillar' muscles (Fig. 62, D). Unlike the preceding types, which are always white in colour, these are yellowish or brownish. They consist of bundles of very large fibrils, 2·5–3μ in diameter, which presumably correspond with the sarcostyles of the other types. In the wing muscles of the bee, which are of this type, there is no sarcolemma;

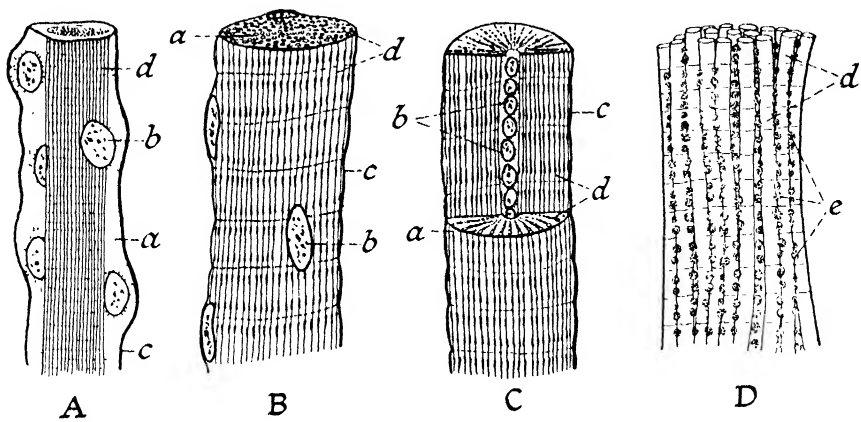

FIG. 62.—Types of skeletal muscle fibres in insects

A, from larva of honey-bee; *B*, from leg muscles of *Melolontha*; *C*, from leg muscles of honey-bee ('tubular muscles'); *D*, indirect flight muscles of honey-bee (a group of sarcostyles from 'fibrillar muscle') (*modified after* SNODGRASS). *a*, sarcoplasm; *b*, nuclei; *c*, sarcolemma; *d*, fibrils or sarcostyles; *e*, sarcosomes.

the fibrils are loosely bound together by the tracheal endings; and between the fibrils are rows of nuclei (sometimes difficult to detect) and of granular oval bodies, perhaps nutritive material, known as 'sarcosomes'.[26, 35] The entire fibre bundle is polygonal in cross section and 90–160μ in diameter.[46]

In all these types each sarcostyle or myofibril consists of alternating isotropic and anisotropic segments; these more or less correspond with the pale and dark-staining discs visible in the fixed tissue. In a given fibre these discs are at approximately the same level in neighbouring fibrils, so that the entire fibre has a banded or striated appearance. The details of this striation vary in complexity in different muscles. In its most elaborate form (Fig. 63) the light disc is traversed by a membrane (the telophragma or Krause's membrane) attached all round the fibre to the sarcolemma, the compartment between adjacent membranes being termed a sarcomere. In the light disc, on either side of the telophragma, there may be a narrow row of dark dots, the accessory disc. And in the middle of the main dark disc there may be a pale stripe, the median

disc or Henson's line, traversed occasionally by a second delicate membrane, the mesophragma.

In the fibrillar type of wing muscles the striation is usually rather less complex than this. But telophragmata are generally present and seem to extend from one sarcostyle to the next, forming a weak union between the adjacent fibrils. In all cases the telophragma seems to be a relatively tough membrane; for under abnormal conditions, when the fibres become swollen, they bulge in the middle of the sarcomeres and are constricted at the telophragmata. This happens both in the types of fibre provided with a sarcolemma and in the individual sarcostyles of the fibrillar wing muscles.[35]

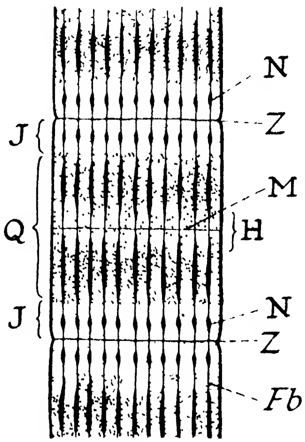

FIG. 63.—Diagram of striated muscle fibre

Fb, fibrilla or sarcostyle; *H*, median disc; *J*, light disc; *M*, mesophragma; *N*, accessory disc; *Q*, dark disc; *Z*, telophragma.

During moderate contraction the anisotropic dark-staining segment is diminished in extent, the pale segment increases somewhat[25]; but in strong contractions both layers become shorter, and then their staining reactions change, so that the dark substance appears to separate and move to opposite ends of the sarcomere, crowding against the accessory discs and telophragmata.[26, 50] In this way the dark bands from adjacent sarcomeres are approximated and form a new dark disc, the contraction disc, so that the striation has the appearance of being reversed.[26, 46]

Muscle insertions—The muscles are united to the cuticle in many different ways. They may end directly on its inner surface, or they may be attached to the base of the epidermal cells and joined to the cuticle by the plasma bodies of these, or by the intermediation of fibrillae passing through them. These fibrillae ('tonofibrillae') can often be followed into the substance of the cuticle, and then appear to be chitinous; but some doubt exists as to whether they are derived from the epidermal cells upon which the muscles are exerting tension, or whether they are continuations of the myofibrillae. If the tonofibrillae are very elongated they may form tendons of insertion; or the muscle may draw the epidermal cells inwards so that they lay down a chitinous stalk which serves as a tendon. In its extreme form this process results in the development of apodemes and internal skeletal structures.[53]

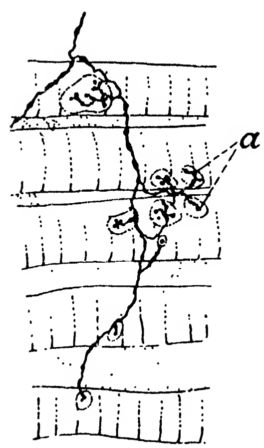

FIG. 64.—Motor nerve endings in oesophageal muscles of *Oryctes* larva

a, Doyère's hillocks (*after* ORLOV).

Nerve endings in muscle—When the nerves reach the ordinary muscles they break up into smaller branches which penetrate between the fibres and have their final terminations anywhere along them. The nerve often ends in a conical projection, the 'end plate' or Doyère's cone (Fig. 64); the nerve fibre penetrates the sarcolemma and breaks up into fine branches with varicose thickenings of all sizes and shapes. These 'end plates'

often contain nuclei belonging to the nerve sheath or neurilemma, and strands of cytoplasm may radiate from them into the substance of the muscle fibre.[36] In the fibrillar indirect muscles of the wings the nerve endings are difficult to find; probably they take the form of delicate branches which end all over the fibre surface.[35] Filiform endings of this kind may occur also in any of the types of muscle.[34]

PHYSIOLOGICAL PROPERTIES OF INSECT MUSCLES

The movements of insects may be very slow, as in the crawling of some larvae, more rapid, as in the running of adult beetles, or excessively quick, as in the wing beats of Diptera or Hymenoptera, which may make more than 200 complete movements per second. We must now consider whether there are any peculiarities about the physiology of insect muscles which will account for these performances.

Absolute muscular power—Some insects are able to lift weights of greater mass than their own bodies, and leaping insects can project themselves great distances through the air. But these achievements are a simple result of their body size. For the power of a muscle varies with its cross section, that is, with the square of one linear dimension; while the volume or mass of the body varies with the cube of the linear dimensions. Consequently, as the body becomes smaller the muscles become relatively more powerful.

The absolute power of a muscle is defined by the maximum load it can raise per square centimetre of cross section. When expressed in this way there is no great difference between the muscles of insects and of vertebrates. Thus the value for man is 6–10 kg. per sq. cm., for the frog 3 kg., for the mandibular muscles of insects 3·6–6·9 kg.,[13] for the hind legs of *Locusta* 4·7 kg.[27] and for the flexor tibiae of *Decticus* 5·9 kg.[48]

Properties of isolated muscles—The properties of the isolated muscles of insects are very similar to those of the skeletal muscles of vertebrates. In

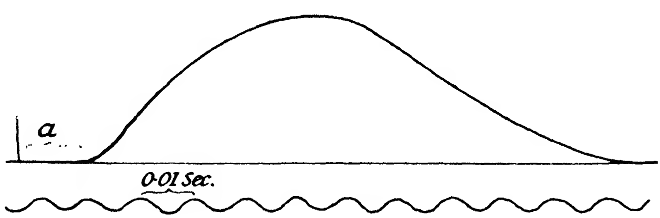

FIG. 65.—Kymographic record of simple twitch in extensor of hind leg of *Locusta* (*after* KAHN)

Latent period (a), 0·012 sec.; total duration of twitch, 0·102 sec.

response to a single electrical stimulus the muscle makes a simple contraction or twitch (Fig. 65). Between the application of the stimulus and the commencement of visible contraction there is a 'latent period' of variable duration; this is followed by a phase of contraction and then of relaxation. Since the contraction is usually recorded mechanically by causing the muscle to raise a lever which writes on a moving drum, the precise form of the twitch is greatly influenced by the mechanical arrangements.

If a second stimulus is applied during the latent period of the first, or during

the early part of contraction, 'summation' occurs and the height of the contraction is increased (Fig. 66).[27] Summation is often very striking in insect muscles, particularly in the more slowly contracting types (it is much more evident in the muscles of *Hydrophilus* than in *Dytiscus* (*cf.* Table 1)).[42] It is

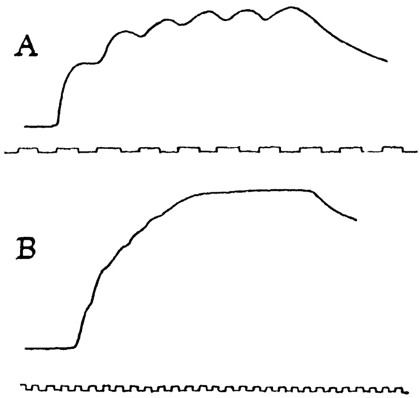

FIG. 66.—Kymographic records from extensor tibiae of *Decticus* at 5° C. (*after* SOLF)

A, stimulated at ½-second intervals, as indicated by the time marker, showing summation; *B*, stimulated at ⅕-second intervals, showing complete tetanus.

well seen in the thoracic muscles of *Aeschna* (Fig. 67); stimuli of subthreshold intensity at the rate of 20 per second have no effect at first, but gradually they produce a contraction which mounts higher with each successive stimulus.[23]

At high temperatures the twitch becomes more rapid; at low temperatures it is slower and relaxation is prolonged so that the form of the contraction wave

FIG. 67.—Kymographic record from wing muscles of *Aeschna coerulea*, given 20 stimuli per second, showing increasing sensitivity to stimuli which at the outset are below the threshold level (*after* HEIDERMANNS)

is changed.[14, 29] When the rate of stimulation is increased above a certain frequency no relaxation occurs; the individual contractions are no longer apparent and a steady state of contraction or 'tetanus' persists (Fig. 66, B).

TABLE 1

Animal	*Latent period in seconds*	*Duration of twitch in seconds*
Bombyx mori larva [32]	—	5
,, ,, imago [32]	—	0·11
Dytiscus [42]	0·017	0·112
Hydrophilus [42]	0·047	0·350
Locusta [27]	0·012	0·10
Locusta [48]	0·013	0·19
Aeschna (wing muscles) [27]	0·015	0·075
Aeschna (wing muscles) [23]	—	0·12–0·13
Frog (gastrocnemius)	0·035	0·12–0·14

Table 1 gives some of the values obtained for the total duration of the twitch and for the length of latent period in some insect muscles. It will be seen that

there is a striking difference between the abdominal muscles in the larva of *Bombyx mori* and those of the imago. In the imago the total duration of the twitch is 0·11 sec.; in the larva contraction is prolonged, recalling that of the smooth muscles of vertebrates; it lasts a minimum of 5 seconds, the phase of ascent never exceeds ½ sec., the rest of the time being occupied in relaxation, and after strong stimulation a persistent 'contracture' may appear.[32] But apart from this the properties of the muscles studied approximate to those of the skeletal muscles of vertebrates (the rapidly contracting muscles of *Dytiscus* have been compared with the white muscles of vertebrates, the more slowly contracting muscles of *Hydrophilus* with the red muscles),[42] and there is nothing in these properties to suggest that they are capable of a specially rapid rate of contraction.

The dragon-fly *Aeschna* makes only about 28 wing-beats per second. But no muscle is known which will undergo maximum contraction and relaxation even at this rate. It has therefore been suggested that during flight the contraction and relaxation of the muscles is not complete, but that they carry out movements corresponding to an incomplete tetanus.[23] It certainly appears as though the flight muscles do require a higher rate of stimulation than most in order to bring about a complete tetanus. Thus the muscles of the frog show a smooth tetanus when stimulated 18–20 times per second, the muscles of the silkworm larva at a rate of 10 per second, and of the silkworm adult at 30–35 per second,[32] the leg muscles of *Locusta* at 15–20 per second [27]; in the leg muscles of *Dytiscus* tetanus is still incomplete at 50 per second [29]; in the thoracic muscles of *Aeschna* the individual contractions may be visible up to a rate of 45 per second in some cases [23]; in *Decticus* tetanus in the leg muscles is complete at 36–50 per second, while in the thoracic muscles at 24° C. fusion of the contractions first takes place at a stimulation frequency of 100 per second.[48] It is possible that the reactivity of the muscles may be still further increased by connexion with the central nervous system.[23]

Chronaxie—An electrical stimulus of infinite duration must be of a certain threshold strength in order to produce a response in nerve or muscle. If the strength of stimulus is increased its duration may be shortened. The 'chronaxie' of Lapique is the duration of the stimulus, expressed in one-hundredths of a second (σ), which is necessary to produce a response when the strength of stimulus is double the threshold. It provides perhaps the most convenient measure of the rapidity of response in muscles and nerves.

The chronaxie in the human biceps is 0·15 σ; in the gastrocnemius of the frog 0·3 σ; in the slowly contracting muscles of *Chironomus* larvae it is 10 σ, while in the imago it falls below 1 σ [4]; in the leg muscles of *Hydrophilus* it is 0·42 σ; in the wing muscles of the dragon-fly *Calopteryx* 0·48–0·72 σ, of *Bombus* 1·84 σ, and of *Calliphora* 0·8–1·12 σ.[19] Thus when measured in this way the flight muscles of insects appear less rapid than the gastrocnemius of the frog. It has therefore been suggested that perhaps the wing muscles do not act directly on the wing, but serve to set in vibration some elastic intermediary structure.[19,23] But at present there is no anatomical basis for this suggestion.

Muscle tonus—In addition to their alternate contractions and relaxations the muscles may enter a state of prolonged steady contracture or tonus. In this state they may support the insect in some characteristic attitude and may have to bear a considerable weight. But the maintenance of this type of permanent contraction appears to demand no measurable increase in meta-

bolism. *Dixippus* shows no difference in oxygen consumption whether it is lying at rest on its back or in a tonic condition with its weight supported by the legs.[8] As we shall see later, this state is dependent on the integrity of the nerve supply (p. 98) and may be greatly influenced by the stimuli from certain sense organs (p. 159). In the muscles themselves (in *Hydrophilus*, flies, &c.) it is associated with electrical waves of small amplitude, present in only a few of the fibres in a given muscle and quite distinct from the electrical responses of much greater amplitude and frequency which accompany voluntary or reflex contraction.[39]

Chemical changes in muscle—The chemical changes which take place in insect muscle during contraction have not been extensively studied; but sufficient work has been done to show that these changes are of the same nature as in the muscles of other groups. During contraction lactic acid is set free: in the thorax of *Aeschna* during rest, lactic acid amounts to 0·283 per cent. of the wet weight, which is about the same level as in vertebrates; after stimulation it is increased to 0·373 per cent.[44] As in most invertebrates, the 'phosphagen' in *Aeschna* [44] and in the blow-flies *Lucilia* and *Calliphora* [2] is arginine phosphoric acid (in place of the creatine phosphoric acid of vertebrates). Hexose phosphate and adenyl pyrophosphate are present in about the same quantities as in vertebrate muscles [2]; in *Deilephila* the latter increases greatly in the actively flying adult as compared with the larva.[24] In short, the muscles of insects seem to show no special chemical peculiarities.

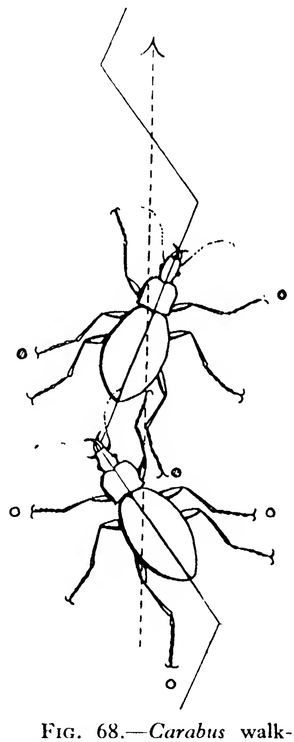

FIG. 68.—*Carabus* walking in the direction of the broken line (*after* v. LENGERKEN)

The continuous line shows the zig-zag course followed by the insect; the circles indicate the legs resting on the ground in the phases represented.

LOCOMOTION

Walking—As was shown by Jean Müller, the insect rests during walking on a supporting triangle, formed by the anterior and posterior limb on one side and the middle limb on the other, while it carries forward the other three legs. The fore leg acts as a tractor; the middle leg serves for support, lifting the body on its own side and together with the hind leg raising the posterior part of the body; the hind leg acts as a propulsor and also turns the body in the horizontal plane. As the result of all these actions the centre of gravity of the insect falling within the supporting triangle of limbs is carried forwards and outwards towards the apex of the triangle until it falls outside this base and its support is taken over by the other triangle of legs; hence the body zigzags from right to left as it advances (Fig. 68). If the insect is caused to walk on smoked paper it is found that the three legs on each side come to lie successively on the same spot. The details of these movements and the mechanics of the muscles which bring them about were fully worked out by Graber.[37]

Crawling of larvae—Some larvae walk by means of their thoracic legs in the same manner as adult forms; but they are often aided by provisional modifications of the abdomen. Thus Carabid, Chrysomelid and other beetle larvae propel themselves forwards by means of eversible 'pygopodia' arising from the terminal segment of the abdomen.[6] Similar structures occur on various Diptera-Nematocera and Siphonaptera larvae. Tenthredinid larvae and the caterpillars of Lepidoptera have well developed prolegs on many of the abdominal segments, and movement then consists of a wave of thickening and shortening running forwards along the body. The anal pair of legs is first carried forwards; the other pairs are moved onward as the wave reaches them; and finally the thoracic appendages are similarly advanced to take up their new position and catch hold.[53]

A large part of the musculature of the body wall in caterpillars consists of small bands running across the many folds in the skin, their sole function being to maintain a steady internal pressure ('turgor muscles'). If the larva

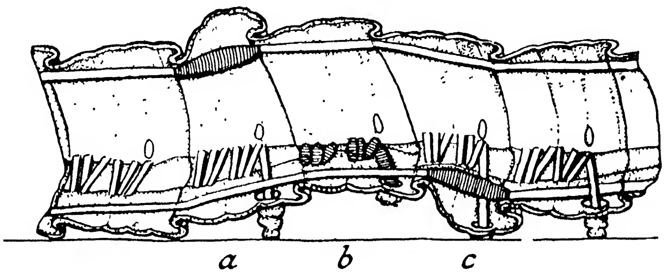

FIG. 69.—Schematic longitudinal section of a caterpillar to show the sequence of muscular contraction during the peristaltic wave

Three segments are active: in *a*, the dorsal longitudinal muscles are contracted; in *b*, the dorso-ventral muscles are contracted; in *c*, the ventral longitudinal muscles are contracted. (*After* BARTH.)

is punctured it shrinks and contracts under the action of these muscles; apparently the fall in pressure provides the stimulus to their contraction. In addition there are the true 'locomotor muscles' (transverse, longitudinal and dorso-ventral muscles of greater length) which do not contract in the punctured larva but are thrown passively into folds. When the caterpillar crawls, the dorsal longitudinal muscles in one segment contract simultaneously with the vertical muscles which lift the proleg in the segment behind, and with the ventral longitudinal muscles in the segment behind that (Fig. 69). This mechanism is variously modified for the looping progression of Geometrids and for the assumption of special attitudes. In all these movements there is necessarily a co-ordination between the turgor and locomotor musculature. The former is highly important in movement; for the extension of any part of the body is brought about through the relaxation of its own muscles while the general internal tension is maintained by the turgor muscles elsewhere.[3]

In other larvae, such as Muscidae, and in motile pupae, legs are entirely absent and progression is effected by peristaltic movements or lateral twisting movements of the body wall combined with friction against the surface due to backwardly directed hairs or spines.

Adhesive organs—Provided the surface on which the insect walks is sufficiently rough, it can hold to it by means of its tarsal claws. If the surface

is too smooth for the claws to grip, it makes use of 'adhesive organs' on the pulvilli, empodia or tarsal or tibial pads (Fig. 70). These organs generally consist of dense collections of tubular hairs ('tenent hairs') with delicate expanded tips, moistened by some glandular secretion. By means of these, many insects can climb on perfectly clean glass; but the mechanism by which they do so has led to much controversy. By some authors they have been considered to act as suckers (as the funnel-shaped organs on the tarsus of the male *Dytiscus* [45, 53] certainly do), being held to the surface by the *atmospheric pressure*, the small amount of fluid present serving merely to make the union between the tubes and the surface airtight.[45, 54] Others have believed the force concerned to be the *surface tension* of the fluid secretion around the margins of the hairs.[43] Others again have supposed that the secretion is a sticky fluid and that it is the *cohesion* of this fluid which holds the insect to the surface.[17] At the present time it seems probable, at least in many cases (*Apis*,[1] *Rhodnius* [21]) that the extremities of the tenent hairs, being very soft and delicate, can be applied so closely to the surface, that in the presence of a small amount of fluid, seizure or *adhesion* takes place and the insect is held by surface molecular forces.[1, 15, 21] Such organs may be of value, of course, not only in clinging to smooth surfaces but for gripping smooth objects such as the bodies of other insects.

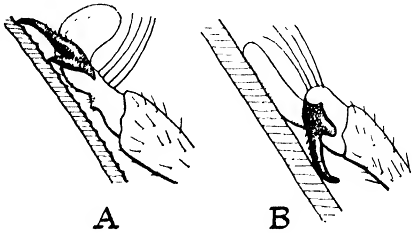

FIG. 70

A, foot of honey-bee applied to rough surface; it holds by the claws. *B*, applied to smooth surface; it holds by means of the adhesive organ (arolium). (*After* CHESHIRE.)

Leaping is usually effected by the sudden combined extension of the tibiae of the hind legs, as in Acridiids, Siphonaptera, *Haltica*, &c. Occasionally it is brought about by a spring and catch mechanism. In Podurids or 'spring-tails' the anal fork is engaged below the 'retinaculum' of the 4th abdominal segment; when the anal fork is forcibly extended it slips out from the retaining catch and projects the insect into the air. A similar mechanism occurs in Elaterid beetles, in which the point of the prosternum is engaged in the fossette of the mesosternum; and in larvae of the 'cheeze skipper' *Piophila*, in which the edge of the anal extremity is seized by the mouth hooks and suddenly released when the larva is in a state of tension.

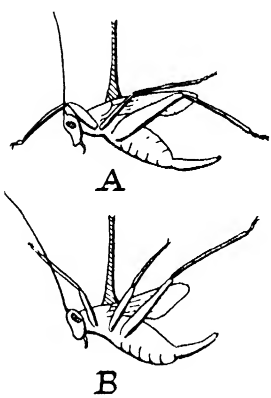

FIG. 71.—Falling reflex in *Meconema varians* (*after* VON BUDDENBROCK and FRIEDRICH)

A, normal attitude of insect supported from the thorax; *B*, attitude adopted when stream of air is blown from below.

Many insects (*Meconema* (Locustidae), *Dixippus*, *Forficula*, &c.) when allowed to fall 20 cm. or more through the air, almost always land on their feet. This results from the reflex assumption of a particular attitude. Legs and antennae are spread out and raised above the body, and the abdomen is curled upwards (Fig. 71). This reflex is induced by the removal of all contact stimuli (*cf.* p. 160) and by the effect of air

currents on the antennae and tarsi. Once the appropriate attitude has been assumed the insect is turned into the dorso-ventral position mechanically by the resistance of the air.[11]

Locomotion on the surface of water—As a rule the cuticle is not readily wetted by water. Consequently, when the insect stands on water the surface tension acts in the opposite direction to gravity. Now the surface area of a body varies with the square of its radius, whereas its volume or mass varies with the cube of its radius; hence the ratio of surface to mass becomes progressively greater as the size of the body diminishes, and the forces resident in surfaces become relatively stronger. Many insects are in fact sufficiently small for surface forces to support the whole weight of the body, so that the insect on the water surface is held up as though by an elastic membrane (Fig. 72). In this it is doubtless assisted, in some cases at least, by glands in the tarsi producing a fatty secretion which enhances the 'hydrophobe' properties of the cuticle and its hairs. The 'pond skaters' or Gerridae spend the greater part of their life resting on the water surface in this way or rowing themselves along by simultaneous backward movements of the hind limbs. Gyrinid or 'whiligig' beetles lie in the water surface with only the polished hydrophobe scutum and elytra exposed; the ventral surface and the highly modified appendages with which they propel themselves along are completely immersed.

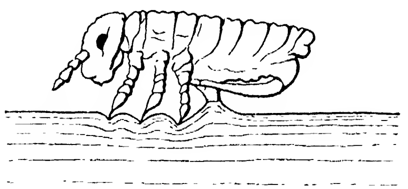

FIG. 72.—*Podura aquatica* standing on the surface of water (*modified after* BROCHER)

The tips of the legs are hydrophobe and depress the water surface. The ventral tube is hydrophile and by means of this the insect can anchor itself in the surface film.

Beetles of the genus *Stenus*, when they fall on water, utilize surface forces in a different way—by expelling from the anal glands a substance which lowers the surface tension, so that they are drawn rapidly forwards like a toy boat propelled by camphor. If deprived of the tip of the abdomen they can no longer move in this manner.[5]

Swimming—Many different mechanisms are used by aquatic insects which swim beneath the water surface. Nematocerous larvae move by lateral flexions of the body, aided in the case of mosquito larvae by 'tail fins' composed of rows of fine bristles. Those larvae which breathe atmospheric air are sometimes raised by the buoyancy of the air they carry; in others (*Corethra* and *Mochlonyx*) the tracheae are modified to form hydrostatic organs by means of which the buoyancy can be adjusted, and they are thus able to maintain the body at any required level (p. 203). The nymphs of Anisopterid dragon-flies propel themselves forwards by forcibly ejecting water from the rectal gill chamber through the anus. The Hymenopterous egg parasites, *Polynema*, &c., are exceptional in swimming under water by means of their wings. The aquatic Coleoptera and Hemiptera swim by movements of the legs. Hemiptera and Dytiscids use their hind legs simultaneously like oars (*Acilius* uses the middle pair as well) so that the sequence of leg movements is completely altered when the insect walks on land; whereas *Hydrophilus* and its allies swim with their 2nd and 3rd pair of legs by the same alternating movement as in walking.

The air stores which these insects carry for purposes of respiration (p. 201) play an important part, also, in the maintenance of equilibrium : if *Dytiscus* is deprived of its air store it goes to the floor of the vessel to seek for air, and if alarmed it swims upwards instead of down.[9]

Flight—In the more primitive insects, Orthoptera, Neuroptera, Isoptera, Odonata, the fore and hind wings are moved independently during flight ; in *Agrion*, for example, when the fore wing is depressed the hind wing is elevated and vice versa (Fig. 73).[52] Whereas in Hymenoptera, Trichoptera, many Lepidoptera, Hemiptera, &c., fore and hind wings are united by various mechanisms to make a functional unit. In Coleoptera, in which the fore wings simply form protective sheaths, they are sometimes held aloft during flight, sometimes, as in *Cetonia*, &c., kept folded over the abdomen. In Diptera the hind wings, and in male Strepsiptera the fore wings have become reduced to richly innervated club-shaped structures, the halteres, whose function in the regulation of flight will be considered later (p. 138).

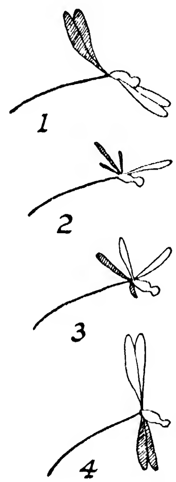

Fig. 73.—Successive positions of the wings of an Agrionid dragon-fly during flight

Fore-wings plain, hind wings shaded. (*From* v. Buddenbrock, *after cinematograph figures by* Voss.)

In all these groups the muscular mechanism of the wing is essentially alike. Flight is effected chiefly by indirect muscles, vertical and longitudinal columns which deform the thoracic capsule by their contraction, the vertical columns elevating the wing, the longitudinal columns depressing it. The complex articulation of the wing gives a wide amplitude to the small and powerful movement imparted to the wing base : in the wasp, for example, the wings vibrate through a sector of 150° (Fig. 74).[33] In addition, there are direct muscles inserted into the wing itself. Of these the most important are the anterior and posterior pleural muscles which pull on the wing base before and behind the pleural fulcrum and so rotate the wing around its long axis (Fig. 75).[40, 47] But in some insects, such as the bee, it is probable that the rotatory movements as well as the vibratory movements of the wing are

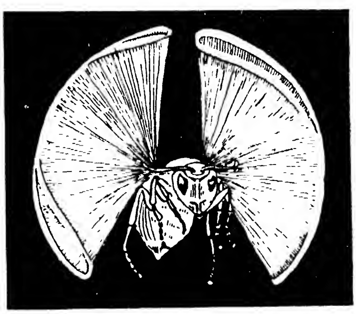

Fig. 74.—Wasp with gilded wing tips hovering in the sun ; to show the extent of the excursions of the wings (*after* Marey)

effected by the indirect muscles, the direct muscles serving only to move the wing in the horizontal plane.[49] In the relatively weak flying Orthopteron

Oedipoda the flight muscles comprise only 8 per cent. of the total body weight, but in strong fliers they make up a far greater proportion : *Musca* 11 per cent., *Apis* 13 per cent., *Macroglossa* 14 per cent., *Aeschna* 24 per cent.[31]

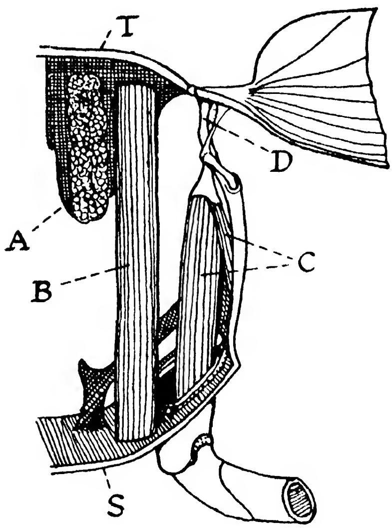

FIG. 75.—Diagrammatic cross-section of a winged segment, anterior view (*after* SNODGRASS)

A, longitudinal dorsal muscle, the indirect depressor of the wing ; *B*, tergo-sternal muscle, the indirect elevator of the wing ; *C*, anterior pleural muscle which deflects the wing forwards ; *D*, the pleural process on which the wing can rotate forward or backward ; *S*, sternum, *T*, tergum.

The movements of the wings during flight have been studied by gilding the wing tips so as to make their trajectory visible,[33] by cinematograph[12, 30, 52] and by observing the positions of the wings assumed naturally in the dead insect.[49] In the hovering stationary insect the wing tips trace in the air an elongated figure of 8, oblique to the long axis of the body (Fig. 76).[30] In some cases, such as *Eristalis*, the downstroke is performed more rapidly than the upstroke.[52] But when the fly is in motion the wings (e.g. in the blow-fly) describe a series of open loops going downwards and forwards and from below upwards and backwards (Fig. 77).[40]

In the course of this movement the wing rotates on its long axis. During the downstroke the surface of the wing looks downwards, whereas it cuts edgeways through the air to some extent during the upstroke, the posterior area being deflected. This twisting movement is due in part to the structure of the wing, which is rigid in front and flexible behind : the wing of a dragon-fly vibrated in a vacuum jar takes on the rotary movement automatically when air is admitted[12] ; but it is chiefly effected by muscular action ; for the wing is concave downwards as it descends, and in *Tipula* the same changes in inclination during the upstroke and downstroke still take place when the wing is reduced to a short stump.[12]

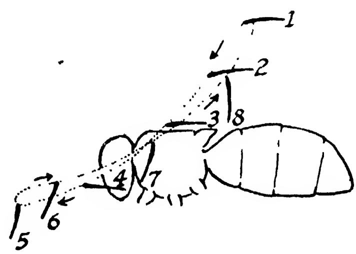

FIG. 76.—Course of the wing tip of a stationary *Volucella* (Dipt.), showing the inclination of the wing at successive points (*after* MAGNAN)

This reversal of inclination during the elevation and depression of the wing produces the same mechanical effect as the revolution of a propeller blade.[47] Each wing, in fact, acts as a propeller which in the stationary insect (as has been demonstrated with Sphingid moths) draws air from above and in front and drives it backwards in a narrow concentrated stream (Fig. 78). The flying insect thus creates a zone of low pressure above and in front, and a zone of high pressure directly behind it.[16] The insect in flight is therefore subjected to the lifting force of the wings directed forwards and upwards, its own mass directed downwards, and the air resistance directed backwards ; it takes up an attitude in the air which is the resultant of these several forces.[10]

Good fliers such as Diptera, Hymenoptera and Lepidoptera can also steer accurately ; they can hover, go sideways or backwards, or rotate around the head or the tip of the abdomen. Steering is effected by unequal activity of the wings on the two sides : the fore wings of *Pieris*, besides their up and down movements, can move forwards and backwards and around their long axis.[51]

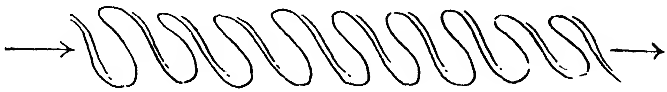

FIG. 77.—The course traced by the wing tip of *Calliphora* during flight (*after* RITTER)

Agrion appears able to modify the inclination of the wing at will ; when going upwards and backwards it lowers the body so that the wings move horizontally, and the inclination of the wing then determines the direction of movement.[12] The bee appears to regulate its forward and backward movement or to hover

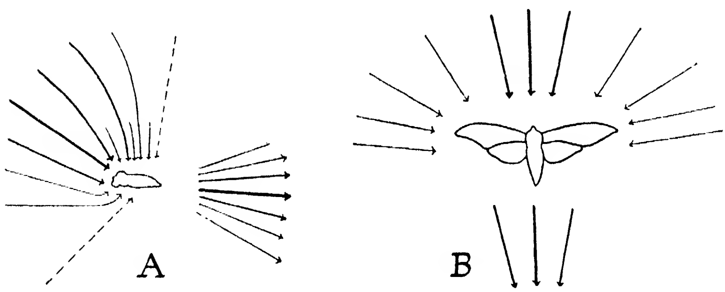

FIG. 78.—Air currents set up by the vibrating wings of a suspended Sphingid

A, side view ; *B*, dorsal view. (*After* DEMOLL.)

in one spot by varying the plane through which the wing vibrates : the more nearly horizontal is the plane of vibration, the greater is the upward drive and the smaller the forward drive (Fig. 79, B–D). It appears to bring about lateral movements by varying the amplitude of vibration on the two sides.

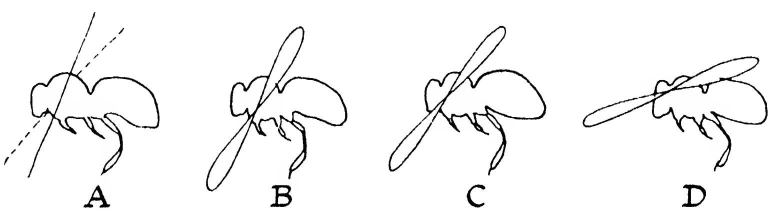

FIG. 79.—Flight in the honey-bee *Apis* (*after* STELLWAAG)

A, a bee turning: the lines show the plane of vibration of the wings on the two sides ; *B*, plane of vibration during forward flight ; *C*, during hovering ; *D*, during backward flight.

The frequency of vibration always remains the same, but the amplitude on one side may diminish until it ceases altogether. Sudden sideways movements are probably effected in this way.[49]

The rate of vibration of the wings varies enormously in different groups. The earliest estimates were derived from the pitch of the sound produced ;

but such values are unduly high because each wing beat gives rise to two pressure waves; so that, for example, the high note of the bee, equivalent to 440 cycles per second, actually represents only 220 wing beats.[22] The rate has been estimated also by tracing with the wing tip on a revolving kymograph or by the cinematograph. The following are some of the values obtained in wing beats per second. HYMENOPTERA: *Apis*, 190,[33] 180–203,[52] 250 [30]; *Bombus*, 130,[30] 240 [33, 52]; *Vespa*, 110.[33] DIPTERA: Tipulids, 48,[30] 44–73 [52]; *Culex*, 278–307 [52]; *Tabanus*, 96 [52]; *Musca*, 190,[30] 180–97,[52] 330.[33] LEPIDOPTERA: *Pieris*, 9,[52] 12 [30]; *Colias*, 8 [38]; *Saturnia*, 8 [30]; *Macroglossa*, 72,[52] 85 [30]; *Acidalia*, 32.[52] COLEOPTERA: *Melolontha*, 46 [30]; *Coccinella*, 75–91 [52]; *Rhagonycha*, 69–87.[52] ODONATA: *Libellula*, 20 [30]; *Aeschna*, 22,[38] 28.[33]

The frequency of beat varies with the load. The normal *Tipula* with wings of 22 mm., with wings cut down to 10 mm., and with wings cut down to 5 mm., shows a ratio in frequency of 9 : 12 : 20.[7] If only one side is shortened, the rate of both is determined by the normal wing.[41] It has already been pointed out that these very high rates of movement cannot be explained by the known properties of the flight muscles. It is perhaps significant that some of the more powerful fliers such as Sphingids are unable to take flight without a period of fluttering during which the muscles of the thorax are warmed up above 30° C. (p. 359), whereas if they have been kept in an incubator at 34° C. they can fly straight away.[18]

Very diverse estimates have been made of the velocity of flight achieved by insects; the following are a few of the values which have been obtained. They are expressed in metres per second (13·4 m. per sec. = 30 miles per hour). HYMENOPTERA: *Apis*, 2·5,[30] 2·5–3·7,[16] 6 [20]; *Bombus*, 3,[30] 3–5.[16] DIPTERA: *Tabanus*, 4,[30] 14 [16]; *Musca*, 2 [30]; LEPIDOPTERA: *Pieris*, 1·8–2·3,[16] 2·5 [30]; *Macroglossa*, 5,[30] Sphingids, 15.[16] COLEOPTERA: *Melolontha*, 2·2–3,[30] 2·5.[30] ODONATA and NEUROPTERA: *Anax*, 8,[30] *Libellula*, 4–10,[16] *Chrysopa*, 0·6.[16] Among these, Sphingids, many butterflies, and locusts can travel several hundred miles without a break.

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Chapter V

The Nervous System

THE NERVOUS system is composed of excessively elongated cells which transmit electrical disturbances or impulses from one part of the body to another. These nerve cells or 'neurones' are derived in the course of development from the ectoderm. Each consists of a nucleated cell body and a long filament or 'axon' (Fig. 80). The axon generally gives off a lateral branch or 'collateral' near its origin and both axon and collateral end in fine branching fibrils, the 'terminal arborization'. Similar fibrils arising from the nerve cell body form the 'dendrites'. Where the axon filaments run freely through the body they constitute the nerves, each filament being invested in a delicate nucleated sheath or 'neurilemma' probably derived from the mesodermal connective tissues. But the greater part of the nerve cells and their processes are massed in a series of segmental ganglia, united by longtiudinal connectives, which constitute the central nervous system. Sensory or afferent neurones convey impulses inwards from the sense organs; motor or efferent neurones convey impulses outwards to the muscles, glands, &c.; and association neurones link the sensory and motor neurones together within the central nervous system.

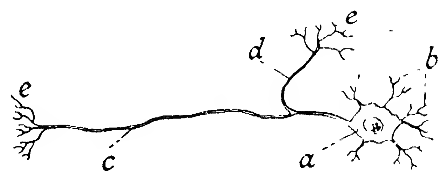

FIG. 80.—Diagram of neurone (*after* SNODGRASS)

a, nerve cell body or neurocyte; b, dendrites; c, axon; d, collateral; e, terminal arborizations.

Neurones of the nervous system—The *sensory neurones* have their cell bodies situated near the periphery. They are generally bipolar, a distal process running to a sense organ adapted to receive some particular type of stimulus (p. 131), and a proximal process running to the central nervous system (Fig. 81, *b*). There is evidence that the proximal filaments from a number of sensory cells may in some cases fuse to form a single compound axon.[32] Indeed, were that not so the sensory nerve from an organ such as the antenna, which may contain many thousands of sensory cells, would be enlarged out of all proportion. It is generally agreed that these peripheral sensory cells are modified epidermal cells, but whereas some authors believe that the central axon is formed by the ingrowth of processes from these cells to the central nervous system,[17, 28, 34] i.e. that they are true 'primary sense cells', others believe that the cell body of the sensory neurone is really situated in the central nervous system and that an axon from this cell grows outward and fuses* with the peripheral 'sense cells' of the epidermis.[48] But

* The view that the axon merely ramifies around the epidermal sense cells[7] has little support.

sensory nerve cells have not as yet been demonstrated in the central nervous sytem.[40]

There are also sensory neurones of a second type. The cell bodies of these are located on the inner surface of the body wall and on the wall of the alimentary canal. They are either bipolar or multipolar and their distal processes, which are often numerous and finely branching, run to the epidermis and somatic and visceral muscles, while their central axons run to the ganglia of the central nervous system (Fig. 81, *c*). Neurones of this type are particularly abundant in soft-skinned larvae, where they anastomose to form a rich nerve net beneath the epidermis, the terminal branches of which seem to end freely on the basement membrane. This system is sometimes regarded as constituting a separate 'integumental nervous system' or peripheral nerve net, homologous perhaps with the nerve net of Annelids.[13, 41]

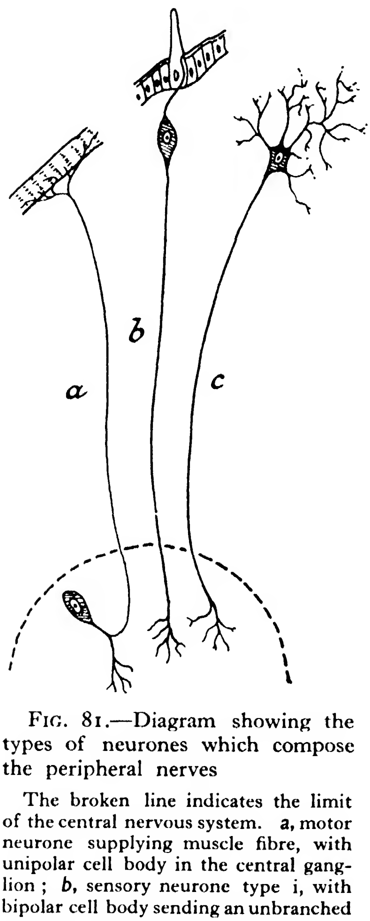

FIG. 81.—Diagram showing the types of neurones which compose the peripheral nerves

The broken line indicates the limit of the central nervous system. **a**, motor neurone supplying muscle fibre, with unipolar cell body in the central ganglion; ***b***, sensory neurone type i, with bipolar cell body sending an unbranched process to sense organ; **c**, sensory neurone type ii, with multipolar cell body giving off branched processes to various tissues.

The cell bodies of the *motor neurones* are situated in the central nervous system. They are nearly always unipolar, and consist of a large pyriform cell body, rich in plasma, located in the peripheral part of the ganglion and connected by a stalk to the 'neuropile'—the central core of nerve-cell filaments (Fig. 81, *a*). The stalk divides into a collateral and an axon filament. The terminal arborizations of the collateral are connected with those of association neurones or sensory neurones. The bundles of axon filaments constitute the motor nerves. These, as we have seen (p. 79), branch around the muscle fibres and end either in Doyère's hillocks or in fine superficial ramifications. As a rule, a given nerve will contain both sensory and motor fibres.

The *association neurones* are similarly placed in the outer parts of the ganglia. Most of them have small cell bodies with nuclei rich in chromatin. Their massed nuclei form the 'globuli' of the outer layers of the ganglia. Some, like the motor neurones, are large, with much cytoplasm containing deeply staining Nissl granules, and with large nuclei poor in chromatin and long thick axon processes.

In addition, the ganglia are penetrated by tracheae and supported by 'neuroglia' cells. These are of two types: epithelial neuroglia cells which form a sheath over the surface and send ramifying processes inwards, and true neuroglia cells with cell bodies often lying in the ganglionic layer and processes branching in all directions.[49]

Fig. 82 shows schematically the chief elements in a typical ganglion as

studied in the nymph of *Aeschna.*[52] Each ganglion contains: (i) Motor neurones, with their cell bodies, collaterals, dendrites and axons; (ii) sensory axons from the periphery; (iii) association neurones of the transverse commissure communicating across the nerve cord or uniting the motor and sensory elements on their own side; (iv) association neurones of the longitudinal connectives, with cell bodies in the lateral part of the ganglion and processes running through the connectives to neighbouring ganglia; and (v) neurones of the unpaired ventral nerve (p. 105).

Each nerve arises by two roots, one conveying the thicker fibres to the dorsal part of the ganglion, the other carrying the finer fibres to the ventral parts. The dorsal root appears to contain the motor fibres: pressure on the dorsum of the ganglion in *Dytiscus*, or pricking in this region, causes motor paralysis without anaesthesia, while the same treatment of the ventral parts causes anaesthesia without loss of movement.[10] And in beetles with immov-

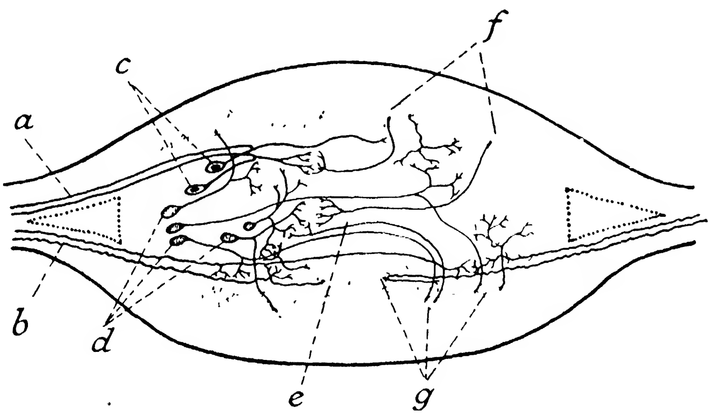

FIG. 82.—Diagrammatic cross-section of abdominal ganglion of an *Aeschna* nymph (*after* ZAWARZIN)

a, dorsal root carrying motor axons; *b*, ventral root carrying sensory axons; *c*, cell bodies of motor neurones; *d*, cell bodies of association neurones; *e*, central neuropile; *f*, dorsal fibre tracts of longitudinal commissures; *g*, ventral fibre tracts of longitudinal commissures.

able wings (*Blaps*, *Timarcha*, *Carabus auratus*) the alar nerve from the second thoracic ganglion is reduced, and only the ventral, presumably sensory, root persists.[10]

Anatomical arrangement of ganglia—Typically, each body segment has a pair of ganglia connected across the mid-line and joined to the ganglia of adjacent segments by paired connectives containing no cell bodies. But there is always some degree of fusion between successive ganglia. In primitive insects such as *Machilis* there are 3 discrete pairs in the thorax and 8 in the abdomen, the last abdominal ganglion being composite. In higher forms the fusion of ganglia is more extensive. The abdominal ganglia are fewer; the first abdominal pair often fuses with that of the metathorax; in higher Diptera the thoraxic ganglia become united into one; in Hemiptera all the thoracic and abdominal ganglia have coalesced to form a single mass.

In the head, the ganglia of the mandibular, maxillary and labial segments have always coalesced to form a suboesophageal ganglion which gives off nerves to these appendages. It is united by a pair of stout oesophageal connectives

with the supraoesophageal ganglion or brain, which lies dorsal to the alimentary tract.

The brain consists of three parts, always fused into a single mass: (i) the 'protocerebrum', which forms the greater part, represents the united ganglia of the optic segment, and innervates the compound eyes and ocelli; (ii) the 'deutocerebrum', is derived from the fused ganglia of the antennary segment; and (iii) the 'tritocerebrum', which is formed by the ganglia of the third or intercalary segment of the head, consists of two small widely separated lobes attached to the dorsal lobes of the deutocerebrum; it innervates the labrum and the anterior part of the gut.

Apart from a few motor elements which innervate the antennae and perhaps others which control the pigment migrations in the eye, motor neurones are almost completely absent from the brain. The nerves it receives are all from the great sense organs of the head; and the nerve cells it contains are nearly

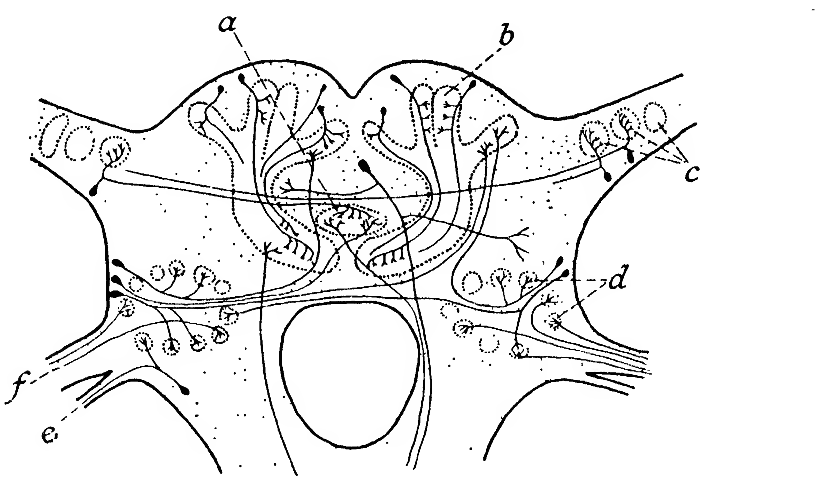

FIG. 83.—Diagram of chief centres and nerve tracts in brain of *Periplaneta* (*after* HANSTRÖM)

a, central body; *b*, mushroom bodies or corpora pedunculata; *c*, optic centres; *d*, antennal centres or glomeruli; *e*, motor nerve of antenna; *f*, sensory nerve of antenna.

all association neurones. As in the other ganglia, these are massed in the cortex; but in addition, in the protocerebrum, there are centrally placed groups of association cells or of their fibres forming the pedunculate or mushroom bodies, central body, ventral bodies, &c., upon which fibres from all parts converge (Fig. 83). The brain is largest in those insects with the most complex behaviour: it composes $\frac{1}{4200}$ of the body volume in *Dytiscus*, $\frac{1}{400}$ in an ichneumon, $\frac{1}{280}$ in *Formica*, $\frac{1}{174}$ in the bee.[26] The central groups of ganglionic cells in the protocerebrum are largest in the social insects: the mushroom bodies comprise $\frac{1}{5}$ of the entire brain in the bee; in the ant *Formica* they are enormous in the workers, in which they make up $\frac{1}{2}$ the brain, smaller in the females and much smaller in the males, although these greatly exceed the workers in body size.[26] They are regarded as higher centres regulating behaviour.

Conduction of impulses in the nervous system—The sensory and motor nerves with the association neurones provide the anatomical basis for behaviour.

The disturbance or impulse which is propagated along them consists in a change in electrical potential, due perhaps to a momentary depolarization of the axon surface, passing like a wave throughout the neurone. These waves succeed one another at a rate which varies with the intensity of the stimulus that is being transmitted; normal contractions in the body muscles are due to trains of motor impulses which rise and decline in frequency as contraction begins and ends.[1] When a group of neurones in a ganglion is discharging impulses simultaneously in this way, they often seem to influence one another so that the potential changes become synchronized in all the axons: the ventral nerve cord of *Dytiscus* isolated from the body shows spontaneous changes in potential of this kind which occur in periodic outbursts from the thoracic and abdominal ganglia, the periods corresponding with the characteristic frequency of the respiratory movements.[1] The same thing is seen in sensory neurones: if the eye of *Dytiscus* is brightly illuminated, all the neurones discharge synchronously and show rhythmic potential oscillations at the rate of 20 to 40 per second, the active neurones tending to stimulate one another until their discharge is synchronized.[2]

Synaptic conduction—The neurones are not continuous with one another. The branched terminations of the axon of one neurone come into intimate association with the dendrites or the collateral arborizations of another neurone to form a 'synapse'. There is increasing evidence in other groups of animals that the electrical disturbance does not itself cross the synapse, but causes the liberation there of some chemical substance (adrenaline, acetylcholine) which sets off a fresh disturbance in the succeeding neurone. Evidence of this has not yet been produced in insects; but however that may be, it is clear that transmission across a given synapse can be influenced by many physiological factors. And since neurones in the central nervous system appear to be connected with one another in almost every direction by way of synapses and intermediary neurones, it is evident that a given impulse may flow along many possible paths.

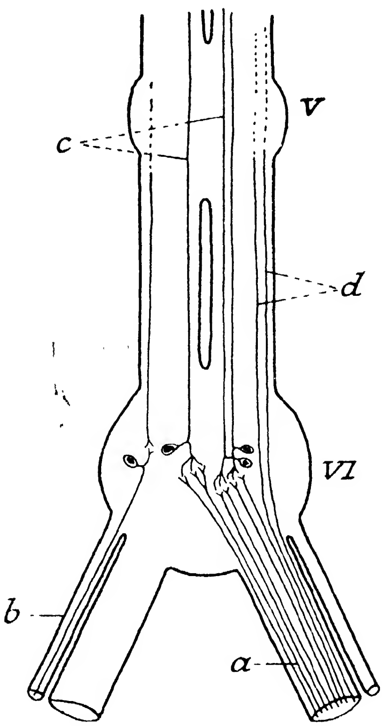

Fig. 84.—Central connexions of cercal nerves in the cockroach (*after* Pumphrey and Rawdon-Smith)

a, cercal sensory nerve; *b*, cercal motor nerve; *c*, giant fibres; *d*, through fibres.

Some indication of the properties of the synapse in insects is given by observations on the cockroach. Each nerve after entering the last abdominal ganglion divides into two roots, one breaking up into fibrils and ending in synapses in the ganglion itself, the other passing straight through to the ganglia in front (Fig. 84).[10] If the nerve from the cercus is stimulated electrically or acoustically (p. 140) and the electrical response in the ganglionic chain recorded, these two types of connexion produce a twofold response. The fibres

which pass through without synapsing produce a small response confined to the same side as the cercus, which persists as long as the cercal nerve is responding. The synapsing fibres produce a response of great magnitude which runs without interruption to the head. This is probably due to stimuli from many fibres of the cercal nerve being collected by way of synapses into a few 'giant fibres' running to the brain. If the cercal nerve is subjected to maximal stimulation above a critical frequency, the synapses become 'fatigued' and the giant fibres can no longer be excited by stimulation of the cercal nerve; although they will still respond if stimulated directly. If sub-maximal electrical stimuli at a low frequency (25 per second) are applied to the cercal nerve, the postganglionic response in the giant fibres gradually declines until the majority of the stimuli fail to get through, although the response in the cercal nerve is unchanged. If the strength or frequency of the stimulation is then increased it will get through. Thus the synapse shows a state of 'adaptation' (due perhaps to a prolongation of the relatively refractory state of the synaptic terminations) in which transmission ceases and is renewed only if the stimuli are strengthened or changed.[33]

Tonus and inhibition—An important function of the muscles, as we have seen (p. 82), is to sustain a constant tonic contraction. In thin-skinned larvae the tonus of the turgor muscles beneath the skin (p. 84) keeps the blood at a steady pressure, and maintains the shape and attitude of the soft body wall. In the turgor muscles this state is dependent on the integrity of the nerve supply from the ganglion of each segment: section of the nerves from a single ganglion in the larva of *Lucanus cervus* [38] or in caterpillars [18, 22] results in immediate loss of tonus and passive distension sharply limited to the one segment (Fig. 85); in *Bombyx mori* larvae a crystal of cocaine applied to an exposed ganglion causes complete flaccidity in the muscles supplied.[31] On the other hand, in *Dixippus*, *Locusta*, &c., the flexor and extensor muscles of the limbs remain in a state of continuous contraction even after separation from the body.[16] It has been suggested that in them the peripheral nerve net may be responsible for this persistence of tonus; but as yet there is no experimental proof of this.[37]

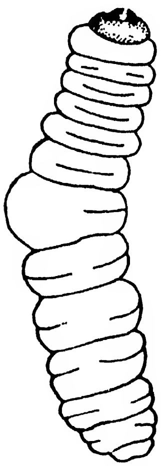

FIG. 85.—Larva of *Cossus* with nerves to left side of the seventh segment cut (*after* BARTH)

When a given muscle (such as the flexor of the limb) contracts, its antagonist (the extensor) must relax. This active relaxation or diminution in tonus is termed 'inhibition'. In vertebrates it is effected centrally by an action upon the neurones which are maintaining tonus. In Crustacea it is brought about peripherally; every skeletal muscle has a double innervation, a thick nerve conveying motor impulses, a thin nerve conveying impulses which break the functional connexion between the stimulatory process and the muscles, and hence bring about inhibition.[13] In insects, inhibition seems to be of this peripheral type. In *Libellula*,[27] *Locusta* and *Meconema* [16] weak electrical stimulation of the nerve of the femur causes extension of the legs with inhibition of the flexor, while strong stimulation causes flexion, the extensors being inhibited. In *Dixippus* the same result is obtained if the thoracic ganglia are stimulated.[16]

Reflex conduction and nerve centres—The simplest type of conduction in the central nervous system will consist in the transmission of impulses by a sensory neurone from a receptor or sense organ to the ganglion, through an association neurone to a motor neurone, and thence to a muscle or other effector organ. Stimulation of the sense organ will thus produce contraction in the muscle. This path of nervous conduction is termed a 'reflex arc' and the response a simple reflex (Fig. 86).

The reflex arc is, in fact, a physiological abstraction; for even in the simplest response the course of conduction must be infinitely more complex, involving as it does inhibition of opposing muscles and compensatory movements elsewhere in the body. Moreover, the course of reflex conduction is not fixed; as the synapses become 'fatigued' or 'adapted' the transmission may be blocked or follow some other reflex arc with a lower threshold; and if the stimuli are excessively strong they may overflow into many paths and produce a discharge of impulses from a large group of motor neurones.

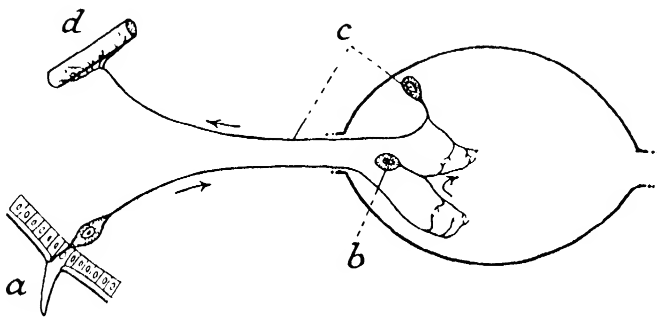

FIG. 86.—Diagram of a simple reflex arc; the arrows show the course of conduction

a, sense organ supplied by a bipolar sense cell which forms the afferent nerve; *b*, association neurone; *c*, efferent neurone with unipolar cell body in the ganglion and axon filament forming the efferent nerve; *d*, muscle.

When a reflex response takes place through a single ganglion, this is said to contain the centre for that reflex. But the occurrence or non-occurrence of the response can be influenced by more remote regions of the nervous system, which may have an inhibitory influence and prevent a response which would otherwise occur, or a stimulatory influence and thus lower the threshold of stimulation necessary to produce the reaction. Where these regions are regarded as having a fixed position they are spoken of as 'stimulatory' or 'inhibitory centres'. For example, the respiratory movements of the gills on the first six abdominal segments of *Cloëon* nymphs in response to oxygen want (p. 198) are said to be controlled by motor centres in their own segments; while these centres are themselves controlled by a stimulatory centre in the 6th abdominal segment and an inhibitory centre in the 2nd thoracic segment.[4] Where movements are made up of an orderly succession of reflexes they are generally considered to be under the control of 'co-ordinating centres'.

Centres in the abdominal and thoracic ganglia—The following are some examples of reflexes obtainable from isolated ganglia, that is, when the nerve cord on either side of the ganglion in question has been severed. A single leg in *Periplaneta* isolated with its ganglion will make a reflex stepping

movement if the tarsus is stimulated by traction [43]; in *Apis* the reflex for insertion and withdrawal of the sting is located in the last abdominal ganglion [8]; the discharge of faecal pellets by caterpillars,[22] and oviposition by the silkworm moth in response to contact of the ovipositor with the surface,[25] are similarly controlled by the last abdominal ganglion; if the skin of a caterpillar is gently stroked with the point of a paint-brush there is a local loss of tonus lasting 10 to 30 seconds—a reflex inhibition with its centre in the ganglion of the segment in question [18]; and the grasping of any object that they touch which is shown by the prolegs of caterpillars is also a reflex obtainable through a single ganglion.[22]

But most of the reflexes shown by insects require the co-operation of several ganglia. In *Lymantria* larvae the movements of turning over when placed on the back, of feeling in all directions when the thoracic legs lack support, or of avoidance of objects brought into contact with the body, are all reflexes involving more than one ganglion [22]; and in *Bombyx mori*, although the last abdominal ganglion by itself will control reflex oviposition, the other abdominal ganglia and the thoracic ganglia ordinarily carry out co-ordinated movements (curving of the abdomen, &c.) during the reaction.[25] The same is true of the complicated movements shown by many insects in which a part of the body is cleaned by one or more of the appendages; movements which are carried out readily by the decapitated insect.[42] For example, on touching the gills or abdominal tergites of *Cloëon* nymphs after decapitation, a wiping reflex can be elicited from the 2nd and 3rd pair of limbs.[4]

Characters of reflex responses—Reflex responses are always purposive; they serve some evident aim in the life of the insect. They are also in many cases so deeply seated in the nervous system that they are carried out even when this purpose cannot be achieved. For example, bugs [21, 42] or cockroaches [19] deprived of their antennae will go through the motions of cleaning them; even decapitated insects may perform these movements.[8] And reflexes may be carried out in the normal way even though they are not performing the function for which they were undertaken. If the antenna of a cockroach is stimulated and it is then offered a bristle or the antenna of another insect, it will clean this and neglect its own appendage.[19]

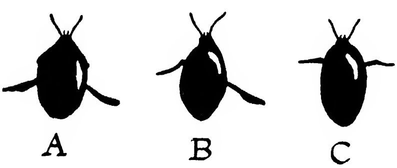

FIG. 87

A, normal swimming movement of *Dytiscus*; both hind legs move simultaneously. *B*, after amputation of left hind leg; left middle leg is used instead. *C*, after amputation of both hind legs; simultaneous movements of the middle pair. (*After* BETHE.)

But reflex responses may be highly plastic. The cockroach normally holds its antenna with one fore leg during cleaning; if the fore legs are removed it may use the middle leg of the opposite side.[19] *Dytiscus* normally swims by means of simultaneous movements of both hind legs; if one hind leg is removed it uses one or both of the middle legs which are normally kept at rest, and if the middle pair is removed the small anterior legs begin to function (Fig. 87). These changes in co-ordination occur equally in decapitated insects.[9]

Similar plasticity is seen in the responses of the insect in nature. Among Cerambycids one individual may clean the antennae with the legs, another with the mouth parts, and if cleaning cannot be effected with one set of

appendages another set is employed.[42] Ants may follow many different procedures in cleaning a given part of the body.[20] And caddis larvae *Neuronia*, which normally build their cases out of fragments of leaves, adopt quite a different procedure when given pine needles and sand or after they have lost the 1st or 2nd pair of legs.[15]

These observations suggest that there are not fixed reflex centres for the various movements but, as was to be expected from the nature of synaptic conduction, the impulses can follow different paths depending on circumstances. Many factors determine what paths shall be followed. Once a given reflex has been set going, conduction in that channel is favoured and the same response may persist a long time [51]; this is termed ‘ facilitation ’. If the end effect of one reflex is prevented mechanically the response may change: when antennal cleaning is prevented in the cockroach the response may be converted into leg cleaning.[19] If stimuli are strong they may flow into other reflex arcs. Thus strong stimuli to the antenna of the cockroach may result in *both* fore legs being employed in cleaning. Or in the earwig, if the leg is gently stimulated with a hair it is cleaned; if it is touched with a needle it is drawn away; if it is pinched with forceps the abdomen is instantly curled to the spot and the pincers brought to bear.[51] And in the dragon-fly, pinching the last abdominal segments results in a generalized reflex, the impulses spreading into many reflex arcs: the abdomen is curved ventrally, the insect sets itself free from its resting place, and the wings begin to flutter.[47]

The nervous system will not always respond to a given stimulus with a given reflex. As we shall see later (p. 157), an important function of the sense organs is to increase the ‘ nervous tone ’ of the central nervous system; that is, to bring it into a responsive state. Reflexes often occur in a definite sequence or chain, each reflex in turn apparently bringing the nervous system into such a state that it will respond to the next. Thus in cleaning the antennae, bugs, flies and other insects will first assume a characteristic cleaning attitude, then clean the legs which are to be used for cleaning, clean the antennae, and finally clean the legs again.[42] The word ‘ instinct ’ applied to behaviour often has very little meaning, but in some cases it seems to be synonymous with this idea of a chain of reflexes.

Co-ordination of walking—The study of co-ordination in walking provides a good example of the nervous regulation of movements. Most insects will both walk and fly after decapitation [13]; the nervous arcs through which co-ordination is effected must therefore all lie in the thoracic ganglia.[44] In *Dixippus*, after section through one of the longitudinal commissures between thoracic ganglia, walking movements remain normal; clearly the nervous paths must cross in such a way that when interrupted on one side they can still pass on the opposite side from one segment to the next; whereas walking is abolished if the cord is cut right through between the meso- and metathoracic ganglia.[12] The same is true of *Mantis*.[36]

The normal method of walking on six legs has already been described (p. 83). If the middle pair of legs is removed in *Dixippus* a new method and a new sequence of leg movements is adopted: the left fore leg is followed by the right hind leg, the right fore leg moves next and then the left hind leg (Fig. 88) [12]; and similar changes in co-ordination appear in *Geotrupes* and many other insects when various legs are amputated.[9] Such changes are so manifold

that it is impossible to assume the existence of pre-formed co-ordination centres ready to control each new type of movement ; each co-ordination must be brought about anew through the interplay of many factors.[13]

One factor in this co-ordination seems to be the tendency for a reflex stimulus to flow into a muscle which is stretched ; in *Libellula* after decapitation, if the leg is flexed stimulation of the tarsus causes extension, if the leg is extended stimulation of the tarsus causes flexion (Fig. 89). But if the stretched muscle cannot respond the stimulus flows into another group.[27] In *Dixippus* failure of contact between the amputated limb and the ground seems to be important, for if the middle pair of limbs is cut short, but the stumps are allowed to come in contact with a little platform attached to the ventral surface of the insect, the four-legged insect still shows the normal walking rhythm.[12]

We have seen that in *Periplaneta* a stepping movement can be induced in a single leg isolated with its ganglion when the tarsus is stimulated by traction. This introduces another factor in co-ordination.[47] Unlike *Dixippus*, *Periplaneta* still shows co-ordinated walking movements after the cord has

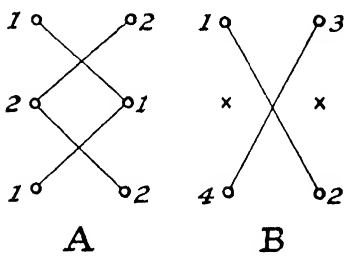

FIG. 88.—Walking movements in *Dixippus* (*after* VON BUDDENBROCK)

The numbers indicate the sequence in which the legs are moved. *A*, normal insect ; *B*, after amputation of the middle pair of legs.

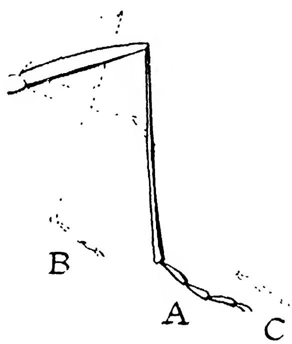

FIG. 89.—Hind leg of *Libellula* nymph isolated with its ganglion

If the tarsus is stimulated when in the normal position, *A*, it is flexed to *B*. If then stimulated it is extended to *C*. (*After* MATULA.)

been cut right through in the thorax. This seems to result from the traction by the anterior appendages evoking reflex walking movements in the posterior appendages, the excitatory action of the anterior ganglia being replaced by excitation from the periphery.[43] The same thing is seen in *Locusta* with the middle legs cut short, and here the movement of the hind leg clearly does not begin until *after* the opposite fore leg, as though stimulated by traction from it.[45] Thus two factors at least are involved in evoking displacement of a given leg in walking : impulses spreading from the ganglia supplying other legs, and impulses from mechanical stimulation of its own sensory endings.

In the crawling of caterpillars [22] and the peristaltic movements of the body in the larva of *Lucanus cervus* [38] co-ordination between the anterior and posterior halves of the insect ceases if the nerve cord is cut through ; it is not affected if only one commissure is cut. Segments paralysed through section of their nerves do not take part in the movement but the co-ordinated wave of contraction is not otherwise interfered with. Hence segmental reflexes are not responsible for the wave of stimulation.[38]

Functions of the subeosophageal ganglion—The suboesophageal ganglion contains the motor centres for the mouth parts which it innervates; but in addition it may influence markedly the motor activity of the entire insect. It does not contain essential co-ordinating centres for movement, since normal walking may occur after its removal.[8] But it exerts an excitatory influence on the locomotor co-ordinating system in the thoracic ganglia, perhaps facilitating conduction in the various reflex arcs.[13] Thus *Mantis* is very sluggish after decapitation, but if the brain is removed and the suboesophageal ganglion left intact it is exceedingly restless; it starts walking on the slightest stimulus and may continue walking for a long time—the suboesophageal centres being perhaps re-excited by impulses from the moving legs.[36] The suboesophageal ganglion has similar properties in *Dixippus* [23]; and it is important in maintaining crawling in caterpillars.[22, 31] But it is not essential for movement in any of these insects, for co-ordinated crawling can be induced after its removal in caterpillars by exerting tension on the anterior segments of the thorax,[18] and *Dixippus*, &c., can be caused to walk by stimulating the thoracic centres by heat.[23] Male Mantids copulate more readily when the suboesophageal ganglion is removed; apparently this ganglion inhibits to some extent the reflexes involved.[35]

Supraoesophageal ganglion—The brain is mainly an association centre. It controls the reflex responses by the rest of the body in accordance with the stimuli received from the great sense organs of the head. The brain is therefore responsible for orientation and for all the more complex forms of behaviour to be discussed in Chapter VIII. It may exert these functions by stimulating or by inhibiting reflexes. Thus in *Mantis* the reflex locomotor activity of the suboesophageal ganglion is subdued when the brain is intact [36]; but without the brain *Mantis* is unable to walk backwards.[36] After removal of the brain in *Hydrophilus*, *Apis*,[8] *Lucanus* larva,[38] &c., reflex responses become exaggerated; responses may be elicited by stimuli which have no effect in the normal insect, and movements (such as cleaning movements) once started may be continued for hours without interruption.[8] Libellulids cannot walk after decapitation because they develop such an active reflex grasping of the surface; a reflex which is normally inhibited by the brain.[47] Females of *Tipula* (Dipt.), after living for some days without egg-laying owing to unsuitable conditions, can be brought by decapitation to immediate oviposition; the inhibitory effect of the brain being removed.[11] In decapitated females of *Bombyx mori* the oviposition response can be evoked repeatedly on pressing the ovipositor: it may be induced before mating, and it persists after all the eggs have been laid. But when the brain is present, oviposition cannot be induced in this mechanical fashion; it is inhibited until after mating. Conversely, it is only in the insect with the brain intact that the normal continued oviposition of a complete batch of eggs takes place.[25] The same type of regulation controls the stinging reflex of the bee.[13]

The brain has an effect also on muscle tonus. Tonus can be maintained by the segmental ganglia [18]; but the general degree of tonus on the two sides of the body is influenced by the brain, each half of which affects particularly the muscles on its own side. Thus if half the brain is removed, tonus is diminished on the side of the injury and the body becomes flexed towards the sound side (Fig. 90).[3, 8, 27, 36] When such an insect walks it moves in a

circle towards the side with the brain intact. After 'pîqure' of the brain on one side in *Dytiscus*, circus movements may persist for months.[10] In *Carabus* and in ants they have been induced temporarily by applying potassium cyanide to one half of the brain.[46]

The mechanism of this circus movement after injury to the brain has been the subject of much controversy. According to one view it is a direct result of the increased tone and contractile power of the muscles on the intact side; these changes affect chiefly the flexor muscles, so that when the insect moves it is carried towards that side.[8, 22] But in *Dytiscus*,[10] *Carabus*, &c.[5] and *Cloëon* nymphs [3] the changed activity of the limbs which brings about the circus movement is not limited to one side but involves the whole organism. In *Dytiscus*,[14] *Aeschna* nymphs,[6] *Phasgonura*, *Dixippus* and many other insects [6] the movement still persists after various limbs have been removed. As in the insect with the brain intact, the reflex paths through which the leg movements are co-ordinated are modified according to the circumstances, but the direction of walking remains the same. From this it has been inferred that the true cause of the circus movement lies in the central nervous system, not in the tonus of the effectors [6]; each half of the brain directing movements particularly towards its own side.[14]

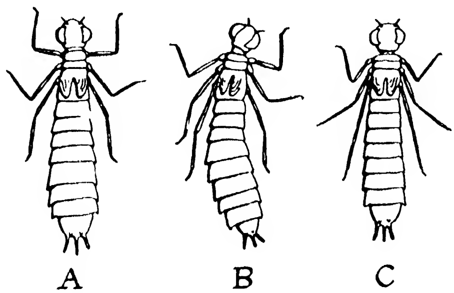

FIG. 90.—Attitude taken up by *Aeschna* larva

A, normal; B, after removal of left half of brain; C, after removal of entire brain. (*After* MATULA.)

VISCERAL NERVOUS SYSTEM AND ASSOCIATED GLANDS

The visceral or sympathetic nervous system of insects is generally described as made up of three parts.

(i) **The stomatogastric system** arises during embryonic development by an ingrowth from the dorsal wall of the stomodaeum which later acquires

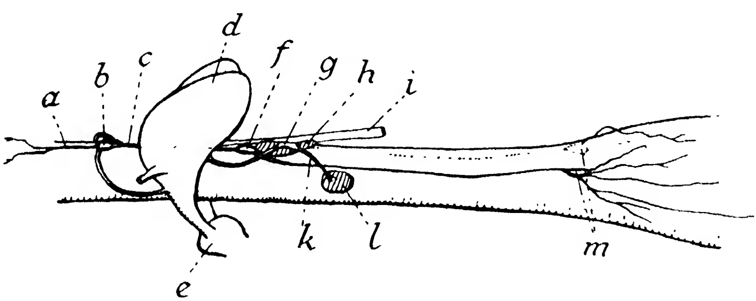

FIG. 91.—Diagram of stomatogastric nervous system (*modified after* DE LERMA)

a, frontal nerve; b, frontal ganglion; c, recurrent nerve; d, supraoesophageal ganglion; e, suboesophageal ganglion; f, hypocerebral ganglion; g, oesophageal ganglion or nervous part of pharyngeal body; h, secretory part of pharyngeal body; i, aorta; k, nerve of corpus allatum; l, corpus allatum; m, stomachic ganglia.

connexion with the brain.[29] It consists firstly of a median 'frontal ganglion', joined by bilateral connectives to the anterior surface of the brain, which sends

back a median recurrent nerve passing between the brain and the oesophagus. The recurrent nerve may end in a paired or single 'stomachic ganglion' towards the hind end of the oesophagus and on its course there is sometimes a median 'hypocerebral ganglion' behind the brain. Behind the brain, also, is a pair of 'oesophageal ganglia', joined by connectives with its posterior surface and sometimes united in the mid-line (Fig. 91).

The system innervates the heart (p. 228) and the anterior segment of the gut (p. 254). It contains both motor and sensory neurones. The motor fibres end in Doyère's hillocks (p. 79); the sensory fibres in bipolar or multipolar nerve cells on the surface of the muscles (p. 137). It doubtless controls the movements of the heart and gut. In *Dytiscus* the frontal ganglion is said to contain the centre for deglutition: swallowing is abolished if it is eliminated; whereas if the frontal ganglion is preserved, swallowing is not affected by destruction of the brain or suboesophageal ganglia.[26]

(ii) **The unpaired ventral nerves** which arise from the suboesophageal and segmental ganglia and give rise to a pair of transverse nerves supplying the spiracles of their segment are generally included in the sympathetic system. They are typically developed in *Aeschna* [52] and in caterpillars. But they are often absent (as in *Dytiscus*) and the spiracles are then supplied from the lateral abdominal nerves.

(iii) **The caudal sympathetic system** which innervates the sexual organs and the posterior segments of the gut is composed of nerves arising from the composite terminal ganglion of the abdomen.

Glands of internal secretion—There is some evidence that the brain itself may produce secretions which are concerned in regulating growth (p. 40). But it is not known where the gland cells in question are located. In the protocerebrum of the bee, cells have been found which contain droplets staining deeply with acid fuchsin; these have been thought to be secreting cells [50]; but similar cells have not been discovered in other insects.[39]

Associated with the visceral nervous system are much more definite organs of internal secretion. The *corpora allata* arise by budding of epidermal cells from the mandibular or maxillary segments. Later these cell nests become separated from the body wall and form compact deeply staining bodies closely connected with the oesophageal ganglia. Each receives a nerve from the corresponding ganglion. In some insects, e.g. many Hemiptera, they fuse to form a single median structure.[29] There is experimental evidence that the secretion from the corpora allata is concerned in regulating growth (p. 45) and perhaps reproduction (p. 393).

It is also claimed that certain organs termed the 'pharyngeal bodies', which may be median or bilateral, and are closely associated with the recurrent nerve and the oesophageal ganglia, are not wholly nervous but contain glandular cells. This conclusion is based on histological evidence in *Dixippus*,[30] *Gryllotalpa*, *Periplaneta*, *Hydrophilus*, &c.[24] There is as yet no experimental evidence with regard to the function of their secretion.

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Chapter VI

Sense Organs : Vision

BEHAVIOUR IS determined, or at least influenced at every step, by the stimuli to which the sense organs are subjected ; while the functions of the sense organs and their powers of discrimination are discovered by observations on behaviour. It is not possible therefore to separate these two subjects completely. But before discussing the general principles in the control of behaviour we must consider the sensory apparatus.

THE COMPOUND EYE

Structure of compound eye—The compound eyes are the chief visual organs of insects. They are made up of a number of transparent facets in the cuticle of the head, each with an elongated light-sensitive structure beneath it (Fig. 92). The facet with its underlying receptor is termed an 'ommatidium' ; the whole collection of receptors in the eye forms the 'retina'. Each ommatidium consists of a distal dioptric part, the 'cornea' with the 'crystalline cone' beneath it, and a proximal receptive part, the 'retinula'. The retinula is made up usually of seven elongated sensory cells (visual or 'retinal cells') containing pigment, each continuous with a post-retinal axon fibre. They are grouped round an optic rod or 'rhabdom' secreted by them collectively. The whole ommatidium is surrounded by a curtain of pigmented cells ; the 'primary iris cells' covering the crystalline cone, and the 'secondary iris cells' investing both the primary iris cells and the retinula (Fig. 98). The proximal extremities of the ommatidia rest on a fenestrated basement membrane through which the nerve fibres and tracheae run ; the nerve fibres pass to the periopticon or outermost tract of the optic lobe of the brain.

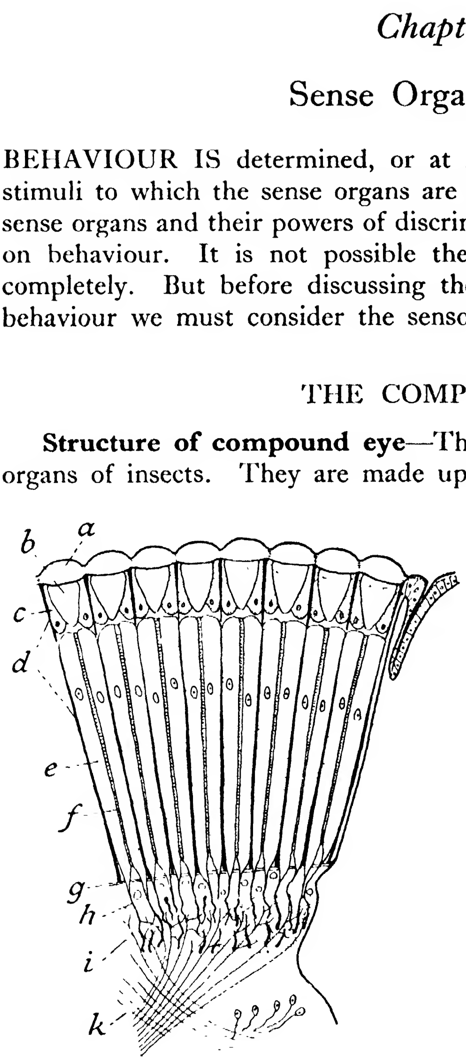


FIG. 92.—Section through margin of compound eye ; semi-schematic (*after* SNODGRASS)

a, corneal lens ; **b**, crystalline cone ; **c**, matrix cells of cornea ; **d**, pigment cells ; **e**, retinula cells ; **f**, rhabdom ; **g**, fenestrated basement membrane ; **h**, nerves from retinula cells ; **i**, lamina ganglionaris ; **k**, outer chiasma.

Optical mechanism of the compound eye—As was originally suggested by Johannes Müller (1829) in his so-called ' mosaic theory ', each ommatidium receives the impression of a luminous area corresponding to its projection on the visual field ; and it is the juxtaposition of all these little luminous areas, varying in the intensity and quality of the light composing them, which gives rise to the total erect image perceived by the insect. The mechanism can be approximately imitated by holding a bundle of tubes with opaque walls in front of a ground glass screen ; an erect image is thrown on the screen, its definition depending on the number of tubes per unit area.[3] In the compound eye the luminous points are formed by the aid of the dioptric apparatus : only those rays which fall normally on the cornea or those which can be brought into this line by refraction will reach the corresponding rhabdom ; those rays which enter too obliquely fall on the pigment and are absorbed. Thus each ommatidium makes use of the rays from only a very small part of the visual field. The erect image formed by the apposition of these points of light has been observed in a number of insects and was photographed by Exner in *Lampyris*.

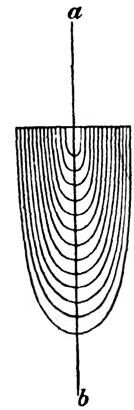


FIG. 93.—Dioptric apparatus of ommatidium of compound eye, made up of superimposed lamellae ; schematic (*from* EXNER)

The dioptric apparatus—As was shown by Exner,[26] the cornea and crystalline cone have rather special optical properties. They have a laminated structure and can be pictured as a system made up of a series of cones with their apices turned inwards and superimposed on one another (Fig. 93). Examination with the microrefractometer shows that in this system the refractive index is at a maximum at the axis and decreases progressively towards the periphery. From the optical standpoint the system can be represented by a series of superimposed cylinders whose refringence increases towards the axis, a system called a ' lens-cylinder '. Let *abcd* (Fig. 94) be a cylinder whose refractive index is maximal at *xy* and diminishes towards the periphery. Let *xm* be a ray falling obliquely on the base *ac*. Within the

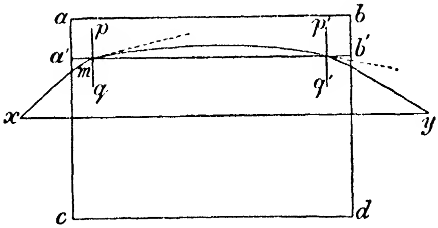


FIG. 94.—Course followed by a ray of light in passing through a lens cylinder (*from* EXNER)

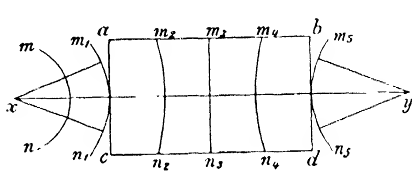


FIG. 95.—Passage of a spherical wave through a lens cylinder (*from* EXNER)

cylinder it encounters the surfaces which separate the layers of unequal refringence, and at each of them (e.g. *a'b'*) it is refracted so that its direction makes a smaller and smaller angle with the axis of the cylinder. Finally it is totally reflected and then follows an inverse course, entering more and more refringent layers, and is brought gradually back to the axis at *y*. An entire spherical wave *mn* emanating from *x* (Fig. 95) will emerge from the cylinder with a concave form

m_6n_6 and converge on y. In considering the passage of light through such lens cylinders the length of the cylinder must be taken into account; and there are two special cases that must be considered in connexion with vision by the compound eye.

(i) When the focus lies at the posterior or retinal base of the cylinder; that is, when the length of the cylinder is equal to the focal distance (Fig. 96). An inverted image will then be formed at the posterior surface; the principal rays coming from the luminous points of the object emerge from the cylinder parallel.

(ii) When the length of the cylinder is twice the focal distance (Fig. 97). In this case, the inverted image of an object placed at infinity is formed midway along the length of the cylinder at yz. The rays continuing from yz in the

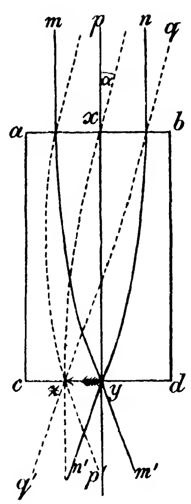


FIG. 96.—Path of rays passing through a lens cylinder of length equal to the focal length (*from* EXNER)

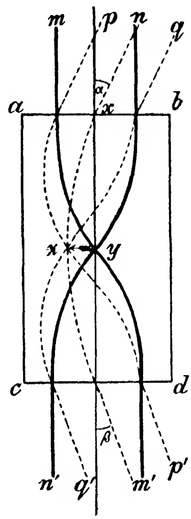


FIG. 97.—Path of rays passing through a lens cylinder twice as long as the focal length (*from* EXNER)

second half of the cylinder follow a course symmetrical with that followed in the first half and emerge at an angle equal to that at which they entered, and diverted towards the same side of the axis of the cylinder.

Exner showed that there are two types of vision in insects corresponding to these two cases.

Formation of images by apposition—In most diurnal insects, Hymenoptera, Diptera, Odonata, many Coleoptera and day-flying Lepidoptera, the cones are surrounded by pigment up to their posterior extremities; they allow the light to emerge only at the central point; and the retinulae are short and placed immediately behind the cones (Fig. 98, A). A reversed image of a small part of the visual field is formed where the retinula comes in contact with the apex of the cone and has been observed in various insects (Fig. 99, A). The arrangement therefore corresponds with case (i) above. But this image has no physiological significance, it merely impresses the retina as a simple luminous point, the apposition of all such points as perceived by the different ommatidia forming the erect image perceived by the compound eye as a whole.

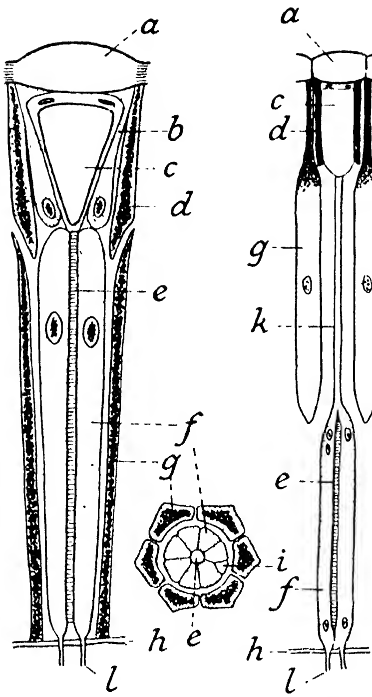


FIG. 98.—Diagrams of the two chief types of ommatidium

A, from eye forming apposition image (*after* SNODGRASS). *B*, from eye of Noctuid forming superposition image (*after* WEBER). *B* is shown in the light-adapted condition with the pigment in the secondary iris cells almost entirely withdrawn into the outer ends of the cells. *a*, corneal lens ; *b*, matrix cells of cornea ; *c*, crystalline cone ; *d*, iris pigment cells or primary iris cells ; *e*, rhabdom ; *f*, retinal or sense cells ; *g*, retinal pigment cells or secondary iris cells ; *h*, fenestrated basement membrane ; *i*, eccentric retinal cell ; *k*, translucent filament connecting crystalline cone with rhabdom ; *l*, nerve fibres.

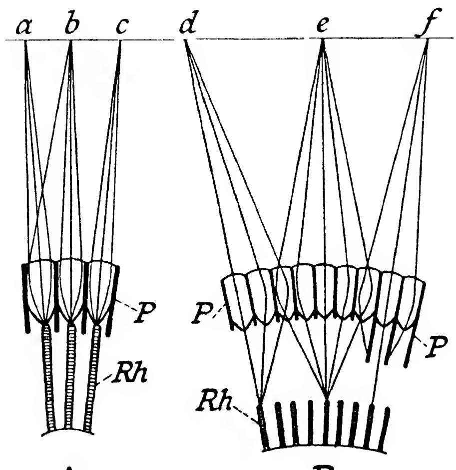
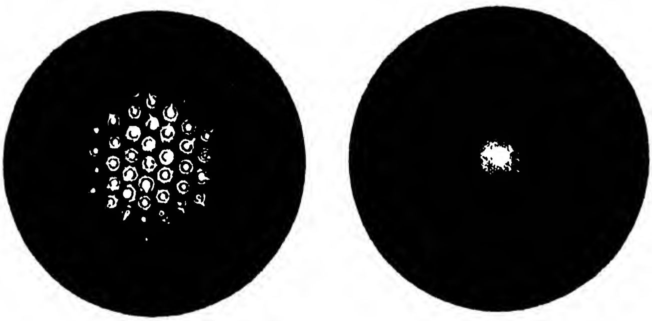


FIG. 99.—Diagram showing image formation by the compound eye (*after* KÜHN)

A, apposition eye ; *B*, superposition eye. *a–f*, luminous points with the course of the rays emitted by them ; *P*, pigment ; *Rh*, rhabdom. At the right side the superposition eye is shown with pigment in the light adapted position ; all rays except those entering the central facet are intercepted.

Formation of images by superposition—In many nocturnal insects, Lampyridae and other beetles, Noctuidae and other Lepidoptera, the ommatidia are greatly elongated. The retinulae do not lie immediately behind and in contact with the cones but are separated from them by a long interval occupied by a non-refractile transparent medium; and the pigment in the iris cells may be concentrated in front between the crystalline cones (Fig. 98, B). The optical system is equivalent to a lens-cylinder twice as long as its focal length. Hence a given rhabdom receives rays not only through its own facet but through neighbouring facets, the rays being refracted by the cones towards the same side from which they have come (Fig. 99, B). For example, if the corneae and adherent cones * of the eye of *Lampyris* are separated from underlying cells and cleaned, and then a minute flame is placed below this group of lenses and the image formed by it is examined with the microscope, it appears as a luminous point (Fig. 100, B). But by focussing the microscope up and down it is seen that the light forming this image does not arise from a single pencil of rays, but from a group of such bundles, each coming through an adjacent facet.



A B

FIG. 100.—Superposition image in *Lampyris* (*after* EXNER)

A, a small point of light as seen through the detached surface of the eye when the microscope is focussed just below the lenses; *B*, as seen when the microscope is focussed at the level of the rhabdoms (*cf.* Fig. 99, B).

So that as the focus is lowered the image point splits up into a number of luminous points (Fig. 100, A). As many as 30 neighbouring ommatidia may unite to concentrate the light in this way upon a single rhabdom. As already described, the image projected upon each rhabdom will be erect. But, again, this is of no physiological significance: the rhabdom receives only a visual stimulus which is presumably the mean of the components of this image. Since each of these elemental images is formed by the superposition of light from a number of adjacent facets, the compound image as received by the entire retina is termed a 'superposition image'. It was this which was photographed by Exner in *Lampyris*; it is seen also in *Cantharis*, *Telephorus*, *Hydrophilus*, Cetoniids among Coleoptera, and in Noctuidae and other Lepidoptera.[26]

Other types of compound eye—There are doubtless many intermediate stages between these two types of image formation. In butterflies, for example, which form retinal images of the apposition type, the image formed at the apex of each cone is erect; so that in some eyes of this type the lens and cone may evidently act together as a lens-cylinder of twice its own focal length.[25] Exner's

* The cone in Lampyrid eyes is not a true crystalline cone (eucone type) but an invagination of the cornea (exocone type).[25]

interpretations apply particularly to 'eucone' and 'exocone' eyes with well-developed cones. But in certain insects the cones are purely cellular and non-refringent ('acone' eyes of Tipulids, *Forficula*, Hemiptera, various Coleoptera) or the cones may be represented merely by a mass of liquid secreted by the crystalline cells ('pseudocone' eyes of Muscids). In these cases the lens system is composed mainly of the cornea and the images are formed by apposition.

The retina—The rod-like rhabdome is composed of highly refractile material; light entering it will therefore be totally reflected from the walls, and thus it forms an ideal structure to receive and conduct light without loss. In the region of the basement membrane there are, in addition, tracheal branches which serve as a 'tapetum' reflecting the light back so that the rays retraverse the rhabdom, and the nervous elements are doubly stimulated. The tracheal tapetum is particularly well developed in Noctuids and other nocturnal insects; in these the tracheae divide up at the base of the retina into innumerable fine branches running a parallel course between the retinulae.[17]

The light presumably brings about photochemical changes in the substance of the rhabdom, the products of which stimulate the surrounding sense cells. It has often been claimed that pigments capable of absorbing light rays are present in the retina and are bleached when light passes through; but this conclusion has generally been based merely on a progressive change which is said to take place in the colour of the light reflected from the eye when it is illuminated [17, 20]; and though such absorbent substances must be present (for otherwise photochemical action could not occur) they have not been conclusively demonstrated.

Pigment movements in the compound eye—The iris cells contain black pigment which absorbs, and pale or coloured pigment which reflects the light. On account of this reflection by the coloured pigment the structure is sometimes termed the 'iris tapetum'. Unlike a true tapetum, however, its function is not to increase the illumination of the retina, but to prevent the entry of the oblique rays.

If the eye of a butterfly is examined with an eye mirror at any part of its surface, it shows a central dark spot surrounded by six smaller spots joined to the first by radial dark lines, and sometimes twelve still smaller peripheral spots. This appearance is termed the 'pseudopupil'. The central spot results from the light being absorbed where it falls on the rhabdom or on the black pigment in the retina, while it is reflected from the iris tapetum. In many butterflies, in the middle of the central black spot there is a small luminous spot, due to the light which falls on the rhabdom not being absorbed but reflected from the tracheal tapetum behind.[22] In certain woodland species of butterflies this central luminous spot disappears when the eye is brightly illuminated. This change is due to the movement of pigment in the cells around the basement membrane (Fig. 101). It takes place very rapidly and may be complete within 6 seconds. Perhaps it serves to keep the illumination of the eye approximately constant with rapidly changing light intensities.[20] Pigment movements may occur also in the apposition eyes of Hemiptera (*Notonecta* and *Corixa*).[8]

But migrations of pigment are much more striking and important in the superposition eyes of nocturnal forms.[71] Most of these may be active also in the day-time, and their eyes possess an arrangement comparable with the mam-

malian iris by which they can be adapted to different light intensities. The retinal pigment is little affected, but the pigment in the primary and secondary iris cells expands and contracts with the intensity of illumination. The cells themselves do not change in shape or position ; the granules of pigment merely become clumped or dispersed. Thus in dim light the pigment in the iris cells is withdrawn upwards and the eye can function as a superposition eye in the manner described above, use being made of all the available light. Whereas in bright light the iris pigment expands downwards beyond the cones, surrounding each retinal element as a black curtain whose extent is proportional to the brightness of the light. In this state the lateral rays are intercepted, each ommatidium receives light only from its own facet, and the eye therefore forms an apposition image (Fig. 99, B). In the living insect examined with an eye mirror in the dark-adapted state, a luminous red reflection from the tapetum is visible from a group of ommatidia ; in the light-adapted state it is visible only from the central ommatidium of the group examined.[22, 46]

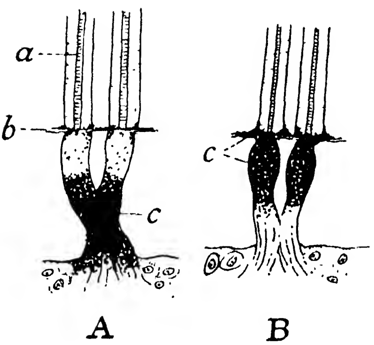


FIG. 101.—Proximal zone of retina in *Vanessa urticae*, showing base of two retinulae

A, in dark-adapted state ; B, in light-adapted state (*after* DEMOLL). a, rhabdom ; b, fenestrated basement membrane ; c, pigment.

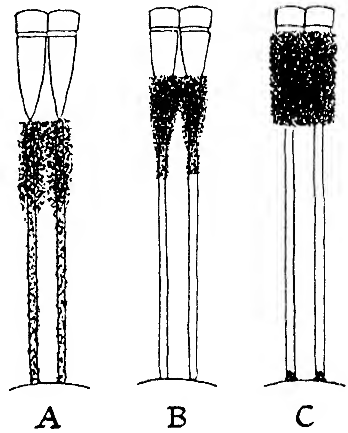


FIG. 102.—Pigment movements in eye of *Carpocapsa*. Semischematic figures of ommatidia, based on photographs (*after* COLLINS)

A, eye completely light-adapted ; B, intermediate stage ; C, completely dark-adapted.

In Mantids the anterior parts of the eye, which are used for binocular fixation, are of the apposition type, the lateral parts are of the superposition type. During the day-time the pigment in the iris cells is expanded ; and since this pigment varies in quantity in different parts of the eyes these appear striped or spotted. At night the pigment retracts in all parts and the banding disappears.[32] In some Lepidoptera the pigment movements are a direct response to changes in illumination ; in nocturnal Lepidoptera the change may be brought about by ultraviolet as well as visible light and takes from 3–17 minutes [68] ; in *Carpocapsa pomonella* (Fig. 102) the movement begins $\frac{1}{2}$ to 1 hour before sunrise or sunset, independently of the length of day, and the change from light to dark adaptation requires about 1 hour for its completion.[18] But apart from this effect of stimulation, many Geometrids, Noctuids, &c., show a diurnal rhythm of pigment migration which persists in complete darkness and does not seem to be due to diurnal changes in temperature, humidity or any other known factor.[46] During

the day such insects have their pigment expanded in the light-adapted position; but on shaking them the pigment immediately retracts to the dark position. This suggests that the expansion of pigment may be simply a phenomenon of 'sleep', and that the rhythm of pigment movement is secondary to a rhythm of general activity.[21]

As regards the controlling mechanism, some authors suppose that the dark-adapted position is maintained by a continuous 'tonus' going out from the brain, and that the movement in response to light is determined by a nervous reflex from the retina. Thus the responses in day-flying butterflies do not occur during the sleep-like state of the insect that has been at rest a long time [20]; the response is abolished by narcosis; and the excised eye always takes up the light-adapted position irrespective of the illumination.[21] Others believe that a photochemical reaction in the pigment cell itself initiates the movement.[18, 71]

Retinal responses—The retina shows an electrical response to illumination such that the cornea becomes negative in respect to the back of the eye. This usually takes place in two waves, an 'A' wave which reaches its maximum 0·10–0·12 seconds after the onset of illumination, followed by a 'B' wave which reaches and maintains its maximum in less than 1 second (Fig. 103). On the cessation of stimulation the potential decays asymtotically until it reaches the original level. A constant response may be obtained with different intensities of illumination provided that the duration of the stimulus is varied in such a way that the product of intensity × duration is constant. This is in accordance with the Bunsen-Roscoe law of photochemistry, according to which the amount of energy (i.e. intensity × duration of exposure) must be constant in order to produce a given amount of photochemical change.[38, 54]

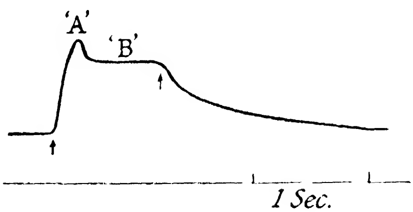


FIG. 103.—Typical form of electrical response to illumination in eye of *Melanoplus femur-rubrum*

'A' and 'B', the two components of the potential wave. The arrows mark beginning and end of illumination (*after* HARTLINE).

The same relation holds in the visual responses of Muscid larvae. The stimulating effect of light on these larvae depends on its brightness (p. 163). If flashes of light following one another at short intervals are employed the effect depends only on the quantity of light received per unit of time. Intermittent light, alternately light and dark, has the same effect as a continuous light of the same average intensity. The eye behaves in this respect exactly as a photographic plate.[72] Applied to the human eye this is known as Talbot's law.

When the eye of *Dytiscus* is stimulated by a bright light, the optic nerve shows rhythmic potential oscillations at the rate of 20–40 per second, showing that all the neurones are discharging simultaneously. But under ordinary illumination this synchronous discharge disappears; the various elements discharge at different rates. It is such differences in the rate of discharge of the separate elements which makes possible the perception of a visual pattern.[2]

Adaptation in the compound eye—The eye is capable of adapting its sensitivity to light over a wide range. This is due in part to the movements of pigment just described. But it occurs equally in insects in which pigment movements are not very obvious. In these it is probably due chiefly to physico-chemical changes in the receptor mechanism of the retina; partly, perhaps, to

central adaptation, that is, a failure of the nervous system to continue to respond to stimuli of a given intensity. Thus in *Eristalis tenax* the sensitivity is judged by reflex turning movements in a beam of light. If this fly after being adapted to a light of 53 metre candles is placed in complete darkness, the sensitivity increases in the course of one hour to a maximum about 21 times greater than at the outset.[23] *Belostoma* (Hem.) shows a similar increase in sensitivity; when exposed suddenly to light of a given intensity it reacts more rapidly the further adaptation to darkness has proceeded.[80] In the bee a reflex movement of the antennae in the presence of moving stripes is used as a measure of the state of photic adaptation. The sensitivity increases rapidly during the first few minutes of darkness and then more slowly until it reaches a maximum in 25–30 minutes, the total increase in sensitivity being about a thousand-fold (Fig. 104).[88]

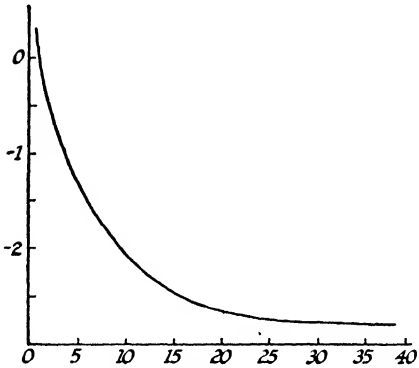


FIG. 104.—Photic adaptation in the honey-bee; relation between threshold intensities and time of dark adaptation (*after* WOLF and ZERRAHN-WOLF)

Ordinate: logarithm of intensity in milli-lamberts. Abscissa: time in the dark in minutes.

Luminosity discrimination—The ability of the insect to discriminate degrees of luminosity in the different parts of the visual field can be judged only from the differences in intensity necessary to produce certain reflex responses. The true threshold of sensation may be considerably lower. In the bee the intensity discrimination as judged by a response to moving stripes (' optomotor reaction', p. 167) is estimated to be about 20 times worse than that of man; with very strong illumination the least detectable difference is not less than 23 per cent.[86] In *Drosophila* it is even poorer; at low illuminations the stronger light must be 100 times brighter than the weaker; under the optimal conditions obtainable at higher illuminations a difference of 2·5 times can be detected.[39]

When the insect is confronted with very narrow stripes, which subtend an angle much less than that covered by a single ommatidium, these will presumably cause a reduction in the intensity of stimulus received by the ommatidium proportional to the amount of the ommatidial field that is darkened. Making this assumption it has been calculated that *Eristalis* will respond when 4·8 per cent. is darkened, *Pieris* 6 per cent., *Apis* 30 per cent., *Coccinella* 26 per cent. But these, again, are thresholds of reflex reactions, not necessarily thresholds of sensation.[14]

The appreciation of luminosity may be influenced by the brightness of the adjacent part of the visual field, that is, by *simultaneous contrast*. For example, *Macroglossa* seeks dark crevices in which to retreat at night and in the autumn; if a series of dark discs of suitable size are set up in a room it makes for the darker ones; and if two of equal darkness are set up side by side, one on a dull grey ground and one on white, the insects make for the latter with some regularity.[56]

Extent of visible spectrum—Insects are characterized by their sensitivity

to the shorter wave-lengths in the spectrum. It was shown many years ago by Lubbock [62] that for ants the visible spectrum extends far into the ultra-violet. Ants which seek the dark will shelter below a flask filled with carbon disulphide, which cuts out the ultra-violet, and avoid a zone covered by a sheet of violet glass which appears quite dark to the human eye. They will choose a light green or yellow zone and avoid a zone covered with deep violet glass. But if a layer of carbon disulphide or quinine sulphate solution is placed over the violet glass (making no perceptible difference to the human eye), they will all collect under the violet shade. If an ant's nest is illuminated with the colours of the spectrum, the ants carry their pupae and deposit them at the infra-red end, just at the limit of our visible spectrum. Whereas at the opposite end they leave vacant a considerable zone beyond our visible range.[62] By covering the eyes Forel [27] showed that they are responsible for this sensitivity to ultra-violet.

Similar conclusions have been reached with many other insects. Moths and other light-seeking insects will fly to glass allowing ultra-violet to pass through, even when it appears opaque to man, in preference to relatively bright blue windows.[64] The honey-bee will respond to light with a wave-length of $297\mu\mu$, at the lower limit of the solar spectrum [9]; and *Drosophila* will give an undoubted response even to $257\mu\mu$.[66] The bee *Trigona* can distinguish patterns made up of white which reflects ultra-violet and Chinese white which does not, although to the human eye they appear homogeneous.[65]

The tracheal tapetum and other parts of the retina fluoresce when the eye is exposed to ultra-violet; they reflect the light at a wave-length falling within the human range. This affords proof that the ultra-violet can penetrate to the retina (in *Sarcophaga* and *Apis* the cornea is quite transparent to a wave-length of $253\mu\mu$) [66]; it also suggests the possibility that in some cases fluorescence may be responsible for the apparent perception of the shortest wave-lengths. It is, however, generally accepted that in most insects the retina is sensitive to the ultra-violet itself.[9, 68]

At the other end of the spectrum there are great differences between different insects. Most insects seem to be insensitive to the deeper shades of red. The honey-bee, for instance, will not respond to light of a wave-length greater than $650\mu\mu$ (on the borders of red and orange).[31, 58] On the other hand, some butterflies (*Pieris brassicae*, *Vanessa urticae*) have an undoubted perception for red, and will visit deep red flowers or red paper models of flowers and may show an actual preference for red.[49] And the fire-fly *Photinus pyralis* will respond to flashes of light from some point between 520–$560\mu\mu$ in the green up to $690\mu\mu$, at least, in the deep red. This is the longest wave-length yet recorded as visible to any insect.[12]

Apparent luminosity—The apparent luminosity of different parts of the spectrum can be inferred only by comparing the efficiency of different wave-lengths in producing those responses which appear to depend on light intensity. Yellow light has the greatest stimulating value of the visible part of the spectrum in causing the ant *Formica* to remove its pupae.[1] Green light is the most effective part of the ordinary dispersion spectrum in causing colour change in *Dixippus* (p. 337); though if light of equal energy is compared the ultra-violet is said to be the most effective and the most luminous region.[75] The most efficient part of the visible spectrum in attracting the bee is the yellow green at about $553\mu\mu$ or $530\mu\mu$, rather close to the region of greatest subjective

luminosity for man. For *Drosophila* the corresponding peak is at $487\mu\mu$ [9]; *Calliphora* $504\mu\mu$; while *Tenebrio* is most readily aroused by light of $535\mu\mu$.[9]

Insects which are attracted to light seem to perceive ultra-violet better than the other parts of the spectrum [64]; and in the case of the bee [9] and *Drosophila* [9] a second peak of stimulative efficiency, four or five times as high as the peak in the visible part of the spectrum, has been described as existing at about $365\mu\mu$ in the ultra-violet. But this conclusion was based on experiments in which light of unequal energy was used over different parts of the spectrum and the relative effect of equal energies determined by calculation. The relative efficiency of wave-lengths of equal energy may, however, be very different for different absolute amounts of energy; so that the validity of conclusions reached by this method is open to doubt.[77] When light of equal energy is used at all parts of the spectrum there is said to be no trace of the high maximum at $365\mu\mu$.[77]

The optomotor reaction, in which the insect responds to moving stripes, alternately dark and light, by turning the head or body, is due to the apparent luminosity of the stripes and not to their colour (but see p. 119); it therefore affords a means of testing the apparent luminosity of different wave-lengths. The conclusions reached by this method agree with those recorded above; the luminosity of various parts of the spectrum is very different in different insects. Some, such as *Apis*, *Mantis*, *Coccinella*, agree with the human eye in its dark-adapted state; others, such as Pierids (which, as we have seen, appear to be more sensitive to red), agree with the light-adapted human eye.[51, 79]

Wave-length discrimination—Flower-visiting insects often behave as though they have a perception of colour. *Bombylius* visiting blue *Muscari* will fly rapidly from flower to flower; they are equally attracted to flowers enclosed in glass tubes or to pieces of blue paper (Fig. 105).[56] *Argynnis* feeding in the field on flowers of blue bugle will turn aside to visit violet or purple flowers made of paper.[49] It was established by Lubbock that bees can distinguish one colour from another; they can learn to associate the finding of honey with blue or orange papers and they will continue to visit the colour to which they have been accustomed although no honey be present.[62] Forel obtained the same results with paper flowers.[28] But these experiments did not exclude the possibility that the colours might be distinguished not by differences in quality but only by their relative brightness. This doubt was removed by v. Frisch by exposing the colour to which the bees had been trained on a chequer board made up of a complete range of grey shades: they recognized their blue or yellow colour among all these greys, even when covered with a glass plate so that smell was excluded; they could *not* be trained to come to any particular shade of grey.[31] The further pos-

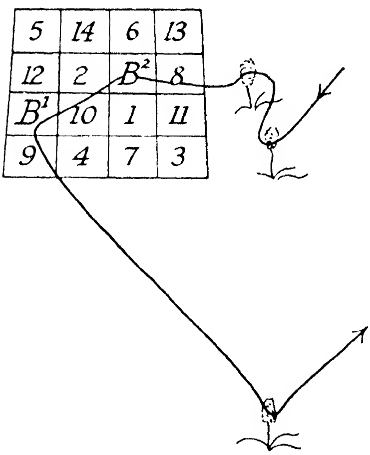


FIG. 105.—Course of flight of *Bombylius fuliginosus* (*after* KNOLL)

The chequer board is made up of grey shades ranging from 1 (very pale) to 14 (very dark), a pale blue square (B^1) and a dark blue square (B^2). The insect flies from blue *Muscari* to the blue squares, but disregards the grey shades.

sibility remained that the coloured papers used might be distinguishable through their reflecting more ultra-violet than any of the greys employed. This was excluded by experiments in which bees were trained to come to bands of spectral light.[58]

Experiments made to discover into how many colour qualities the spectrum is divided have shown that there are wide differences between insect and man and between different insect species. Bees tested by training to bands of spectral light appear to distinguish four regions in the spectrum: 650–500$\mu\mu$ (red, yellow, green); 500–480$\mu\mu$ (blue-green); 480–400$\mu\mu$ (blue and violet); 400–310$\mu\mu$ (ultra-violet). In their perceptions at the red end of the spectrum they approximate to man with red-green colour-blindness.[58] They can distinguish certain red flowers, such as *Papaver rhoeas*, but that is only because these reflect ultra-violet; bees trained to visit such flowers are equally attracted to a black glass emitting ultra-violet rays invisible to man.[61] The ultra-violet region is probably perceived as a true colour; and from this it follows that reflected daylight probably appears colourless to the bee only when its composition approximates to sunlight; if deficient in ultra-violet, 'white' light will appear coloured.[43] Bees experience also the phenomenon of simultaneous contrast: a grey area surrounded by yellow appears blue and is visited by bees trained to this colour; surrounded by blue it appears yellow. Blue and yellow therefore form complementary colours; and probably blue-green and ultra-violet are also complementary.[58]

Butterflies offered paper flowers of various gay colours obviously prefer these before grey paper flowers. If the number of visits to different colours are counted, *Vanessa urticae* shows two maxima, one in the blue-purple group and one in the yellow-red group; green and greenish-yellow are unattractive. By training for blue at the expense of yellow and red the blue peak of the curve can be relatively increased and the red-yellow peak decreased or vice versa. *Pieris brassicae* in search of food shows a spontaneous choice of blue and purple papers and, to a less extent, red and yellow; it disregards green, blue-green and grey.[49] But the egg-laying female of the same species shows a peculiar 'drumming' reaction of the fore legs with which it tests the leaves of the food plant; and for this reaction it chooses emerald green to greenish blue papers, yellow and pure blue are neglected (Fig. 106); though it will sometimes show the 'drumming' reaction at the margin of a purple paper—evidently in response to the green contrast colour.[52] Thus, unlike the bee, *Pieris* shows a definite discrimination between yellow and green; in this and in the discrimination of red its colour vision approximates more closely to that of man.

Deilephila can discriminate the blue-violet-purple group of colours of the flowers on which it feeds, from the yellow-green group. It feeds in the dusk and it is able to recognize these colour differences in light so dim that the human eye can no longer see any colour at all.[56] *Macroglossa* shows the same preference for blue flowers during feeding; but during oviposition it is attracted to yellowish-green flowers and plants.[56] Training experiments show that *Aeschna* nymphs can distinguish yellow from violet and grey.[57]

Another method of testing for colour perception is by the use of the optomotor reaction (p. 167). Stripes of a certain blue colour x are alternated with grey stripes of different shades until a grey shade y is found which is equal in luminosity to x and causes no response when the stripes are moved in front of

the insect. A yellow colour z is then found which will give no response when alternated with the grey shade y. If now the blue x is set beside the yellow z a response is given : evidently there is a perceptible difference in quality between these two. By this method it has been shown that a colour vision exists in the beetles *Chrysomela*, *Agelastica* and *Geotrupes*, in the fly *Fannia*, and in many Noctuids and Geometrids. The conclusions in the case of the bee agree with those obtained by other means. The plant-feeding Chrysomelids clearly distinguish green tints from one another as well as yellow and orange from blue-violet and green.[78] The nocturnal *Dixippus* and the bug *Troilus* on the other hand appear to possess no colour vision [78] ; a conclusion which has been confirmed in the case of *Dixippus* by other means.[48] *Trialeurodes* flies only to yellow or green-yellow surfaces. but this seems to be merely because they provide the medium light intensity that is preferred.[84]

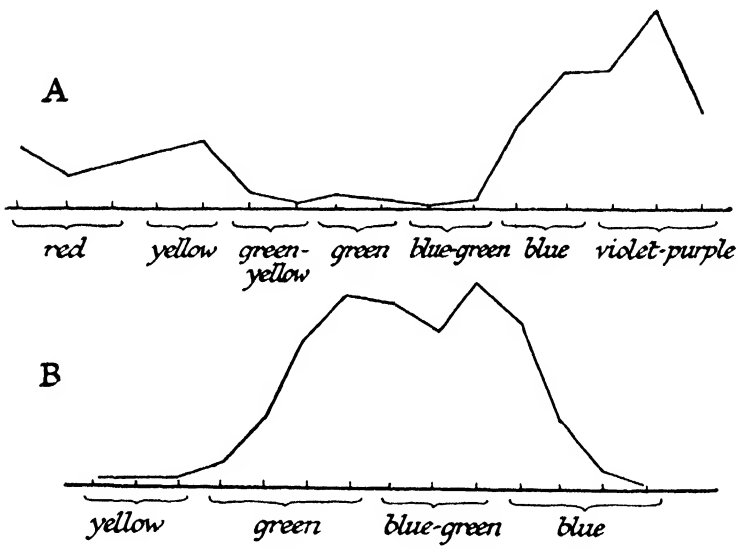


FIG. 106

A, chart showing the relative number of visits of *Gonepteryx rhamni* to different coloured papers during the feeding phase ; *B*, chart showing the number of 'drumming reactions' shown by *Pieris brassicae* females on coloured papers during the egg-laying phase. (*After* ILSE.)

Whether there is a separate mechanism in the eye for perceiving colour is not known. In many insects one of the retinal cells in each ommatidium differs visibly from the others, (Fig. 98, *i*), and the axons from certain of the retinal cells differ in their central connexions. It is possible that these differences correspond with differences in function ; but at present this is pure hypothesis.[37, 41]

Perception of form—The retinal image, as we have seen, consists of a mosaic of points of light of varying luminosity and colour ; a mosaic which will be coarse or fine depending on the number of facets per unit area. If the insect responds to changes in this visual pattern it may be regarded as having some degree of form perception.

In many insects such perception seems to be of a very elementary kind. If the fly *Eristalis* is allowed to crawl along between two walls, one plain and the other with vertical stripes, it always inclines towards the striped side, and the

more so the more broken up the pattern is—unless the stripes become so fine that they can no longer be resolved by the eye.[13] *Coccinella* placed on a white ground with black stripes will follow the stripes and turn corners when they are angulated. This response seems to be best developed in phytophagous insects and is perhaps associated with their habit of following stalks and branches in their search for leaves.[82]

Bees can be trained to associate certain black figures on a white ground with the presence of food. The contrast of such figures against the background, the extent to which the figure is divided up, and the arrangement of the contours, are all properties which seem to be independently perceived.[42] But such figures as triangles, squares, circles and ellipses, which are well within the capacity of the retina to differentiate and which appear very different to our eyes, are apparently not distinguished by the bee. On the other hand, it can readily distinguish from these any figures which are markedly broken up into black and white areas—rows of stripes, chequers, flower-like patterns, &c. (Fig. 107). It shows a natural preference for such divided figures, and this preference cannot be overcome by training: the bee will learn to associate the presence of food with a given figure only if this has a more sub-divided form than the control figure[90]; a simple disc is visited only after all other more divided figures have been removed.[42] There is in fact a direct proportionality between the richness of contour in the figures and the number of times they are visited by bees.[90] In butterflies, also, the choice of colour patterns is favoured if they have divided contours.[50]

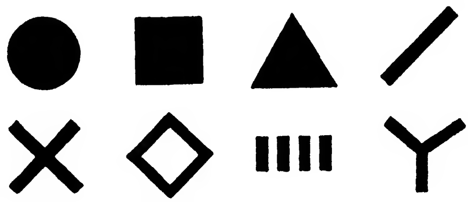


FIG. 107.—Perception of form by the bee

Figures of the upper row are indistinguishable from one another. The same applies to the lower row. But figures of the lower row are readily distinguished from those of the upper (*after* HERTZ).

From these observations it would seem that the choice of forms by these insects is dependent merely on the frequency of change of retinal stimulation; and that they cannot recognize figures by any properties of configuration.[87, 90] In other words, that the perception of form is little more than a perception of different degrees of flicker.[87] Thus, in nature, bees settle more rapidly on flowers if these are being shaken by the wind, or on models of flowers which are artificially kept in motion; they are attracted to moving stripes, and the more rapidly these are moved the greater is the attraction.[87]

But in addition to this type of response it is certain that some insects have a more integrated perception of the form or arrangement of objects in the outside world. Whereas *Aeschna* nymphs will snap repeatedly at any moving object [5] and hunting wasps will fly at nails hammered in a wall, mistaking them for flies,[28] *Aeschna* adults, which will turn towards a paper pellet thrown in the air, attracted by its movement, will instantly turn away from it when its image is perceived.[5] Bees and ants utilize visual landmarks in finding their way back to the nest (p. 173). The wasp *Philanthus* locates the entrance to its nest by the arrangement of visual marks around it, and by moving these it can be led astray at will.[81] And bees, which can recognize their hive by coloured marks at the entrance,[31] can appreciate the difference if a given colour is on the right or the left of the entrance hole.[30]

Visual acuity—Fig. 108 shows the well-known photograph obtained by Exner (1891) of the retinal image of *Lampyris splendidula.* It represents a window with a letter R on one pane, at a distance of 2·25 metres, and a church tower beyond. The degree of resolution which this represents has been estimated to correspond with a visual acuity $\frac{1}{60}$ to $\frac{1}{80}$ that of the normal human eye.[67]

The resolving power of the compound eye must depend in the first place upon the number of retinal elements, that is, the number of facets. In *Dytiscus* there are 9,000 facets in each eye ; in *Melolontha* 5,100 ; in *Necrophorus* 3,500 ; in the winged male of *Lampyris* 2,500, in the wingless female 300 ; in the workers of the ant *Solenopsis*, which live almost always underground, 6–9, in the male of the same species which pursues the female high into the air, 400 [22] ; in *Musca* there are 4,000, in *Odonata* 10,000–28,000.[53]

FIG. 108.—Photograph of retinal image in *Lampyris splendidula* (*after* EXNER) It shows a window with a letter R on one pane and a church beyond.

But more important in determining the resolving power will be the angular extent of the visual field that is covered by each element, that is, the ommatidial angle. The ommatidial angle in *Apis* is 1°, in *Forficula* 8° ; *Forficula* will obtain only a single point of light from an object which *Apis* will resolve into 64.[15] As the ommatidial angle becomes smaller, although the resolving power will increase, less light will enter each facet and hence the image will be much less luminous. But if the eye is larger the same ommatidial angle will entail a larger facet size and hence a brighter image. Or, conversely, for the same brightness of image, the larger species can have a greater number of facets, with a corresponding increase in resolving power. For example, in the three Lamellicorns *Polyphylla fullo*, *Melolontha vulgaris* and *Phyllopertha horticola* the total angle covered by the eye is about the same, and the size of the facets is about the same, but the number of facets is 12,150, 5,475 and 3,700 respectively ; the large *Polyphylla* has three times the facet number of the small *Phyllopertha.*[15] The facet size does in fact remain much more nearly constant than the size of the insect or its eye. The diameter of the facet in *Culex pipiens* is 16μ, in *Melolontha vulgaris* 20μ, in *Periplaneta americana* 32μ, in *Anax formosus* and *Libellula depressa* 40μ ; their surface area thus ranging between about 256μ^2 and 1600μ^2.[15] In some insects the facet size is greater in certain parts of the eye : in males of *Tabanus* the facets are larger over the upper and anterior parts ; in *Simulium* and *Bibio* the two areas of different sized facets are distinctly separated ; and in *Gyrinus* and *Cloëon* they are quite remote.[53]

In nocturnal insects, as we have seen, the luminosity of the image is increased by superposition of stimuli from adjacent facets (p. 112). Presumably this

must involve a large sacrifice in the sharpness of the image. In Mantids the lateral parts of the eye form superposition images while the anterior parts, used for binocular fixation, form apposition images.[32] In superposition eyes the curvature of the eye surface is usually regular and spherical, as it must be if it is to function correctly. But in apposition eyes the curvature varies in different parts and then the flattest regions, where the angle between adjacent ommatidia is smallest, will be regions of most acute vision.[73] In the bee the ommatidial angle is twice [73] or three times [7] as great in the transverse plane of the eye as it is in the longitudinal plane. This will have the same effect as astigmatism in man. And there is some experimental evidence that the visual acuity of the bee is in fact greater in the vertical axis than in the horizontal.[7, 40]

Where a single object or point of light forms an image on the retina it can be more or less clearly seen even though the image does not cover a single rhabdom completely. In *Formica rufa* the ommatidial angle is 3·5°; but it will respond to a suspended sphere subtending an angle of only 2·5°.[45] (In man the least detectable angle is about 1′.) The same applies to the perception of a narrow stripe; this will slightly reduce the illumination of a succession of ommatidia across the eye, and if there is sufficient contrast between the stripes and the background, so that the stimulus upon adjacent ommatidia exceeds their contrast threshold, it will be perceived.[42] Thus newly hatched *Dixippus* nymphs can perceive stripes rather narrower than the ommatidial angle of $7\frac{1}{2}°$ [55]; though in *Musca*, with an ommatidial angle of 3°, the narrowest stripe which appears to be perceived subtends an angle of 5°. [33]

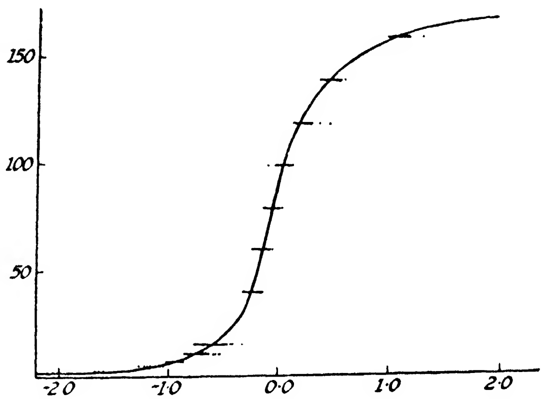


FIG. 109.—Relation between visual acuity and intensity of illumination in the honey-bee (*after* HECHT and WOLF)

Ordinate: units of visual acuity. Abscissa: logarithm of intensity in millilamberts.

The bee [40] and *Drosophila* [39] make characteristic reflex responses when a striped pattern is moved in front of them. Such movement can be perceived only if the pattern is resolved and if the differential illumination of the two components is detectable. It therefore provides a means of testing both intensity discrimination (p. 116) and visual acuity. By this method it has been shown that the visual acuity varies with the logarithm of the intensity along the same type of sigmoid curve as in man (Fig. 109). At low intensities the resolving power is very poor; under optimal conditions it is judged to be something like $\frac{1}{100}$ that of man in the case of the bee, and $\frac{1}{1000}$ in the case of *Drosophila*.[39, 40] The falling off of both acuity and intensity discrimination at low illuminations is attributed by some to the different ommatidia having different thresholds of response: at low illumination only the most sensitive are functional.[39, 40] By others it is attributed to a nervous coupling of groups of ommatidia to form a new unit when the illumination is low.[16]

That leads to another factor in visual acuity: the extent to which the indi-

vidual ommatidia are normally coupled to a single nerve. From histological studies it has been concluded that in *Calliphora* the coupling of ommatidia is much less pronounced in the anterior region of the eye. Apart from considerations of ommatidial angle, this will therefore constitute a region of most acute vision comparable with the fovea centralis of vertebrates.[7] Possibly there is less coupling in insects with apposition eyes, for in these forms the optic ganglia show more massive development.[73]

Movement perception and flicker. An eye of low resolving power like the compound eye of insects will serve to detect changes in the visual field, that is, movements of objects, rather than their form.[26] *Aeschna* nymphs cannot be induced the snap at an object unless it is moving ; the males of many Lepidoptera are not attracted to the females unless these are in motion. Insects also experience the phenomenon of 'induced movement' : *Aeschna* nymphs will snap at stationary objects if a striped screen is moved behind them ; or sometimes they will snap at such objects as soon as the screen ceases to move.[33] On the other hand, flies do not respond to stripes which are made to appear and disappear in succession round a screen, which gives the semblance of movement (stroboscopic movement) to the human eye.[42]

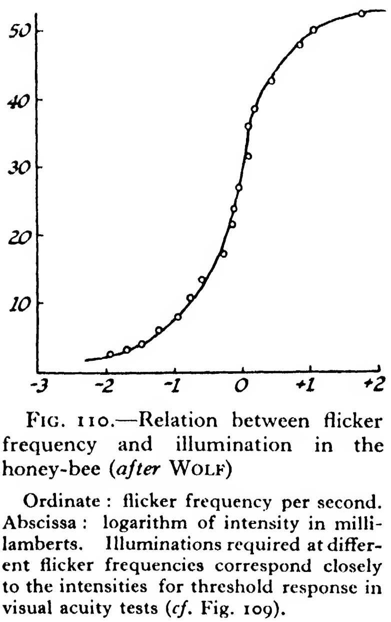


FIG. 110.—Relation between flicker frequency and illumination in the honey-bee (*after* WOLF)

Ordinate : flicker frequency per second. Abscissa : logarithm of intensity in millilamberts. Illuminations required at different flicker frequencies correspond closely to the intensities for threshold response in visual acuity tests (*cf.* Fig. 109).

The optomotor response (p. 167) is the result of a perception of movement ; and this response can be used to determine the minimum dark interval between two light impressions which is necessary if the two are to be separately perceived. It is estimated that in the case of *Aeschna* nymphs this minimum interval is 0·0165 seconds ; at higher rates the images fuse. In other words, the 'flicker threshold' is about 60 stimuli per second.[76] In *Anax* nymphs a similar value is obtained [19] ; in the bee a value of 55 flickers per second [87] ; the corresponding figure in man being 45–53. In *Anax* the value varies with the intensity of illumination and the temperature ; it is regarded as a sort of discrimination of intensity between the effect of the flashes of light and the after effect of these flashes during the intervals of darkness (Fig. 110).[19]

Distance perception—Since the eyes of insects are not independently movable, and therefore have no fixation plane upon which they can be converged, stereoscopic vision such as occurs in man cannot exist. But, the eyes being fixed, the distance of any point in the visual field will be determined by its position on the points of intersection of the individual ommatidial axes.[26] The visual field in *Notonecta* amounts to about 246° in the horizontal plane, 94° of which is covered by both eyes ; in the vertical plane the field is 360°, of which 120° is binocular dorsally and 80° ventrally.[63] The simultaneous and equal illumination of corresponding points of the retina is probably the chief factor in the perception of distance (Fig. 111).[22] In *Aeschna* nymphs this mechanism

is much more efficient when the point of intersection is on the median axis; and the insect, in fact, always turns so that this axis is directed towards any moving object which attracts attention. In the medial region of the eye the visual angle of the ommatidia becomes progressively smaller (1° 12′); this will aid definition as the object is approached. In *Aeschna*, snapping first takes place when the object is within range of the labium[6]; in adults of *Cicindela*, which can observe their prey at 12–15 cm. distance, no attempt is made to seize it until it comes within reach of the mandibles at the point of intersection of the innermost visual axes.[29]

By this mechanism the judgement of distance is absolute and not relative to the surroundings: *Aeschna* is equally accurate in its judgements in a dimly lit tank with plain black walls. The localization is purely visual, for it is effective through glass.[6] In *Agrion* the tactile perceptions of the antennae are normally important, also, in the judgement of distance, but 14 days after the antennae have been removed, the nymphs can seize their prey with the same precision as the normal insects.[4]

Appreciation of distance is seen, also, in the ant: the visual angle which a moving object must subtend in order to produce a response in *Formica rufa* is smaller if the object is close. This difference disappears when one eye is blackened.[45] *Aeschna* nymphs and *Cicindela* adults likewise lose most of their power of judging distance after unilateral blinding. But a somewhat indefinite monocular localization still persists; dependent perhaps on the greater definition by the ommatidia at the inner margin of the eye.[6, 29]

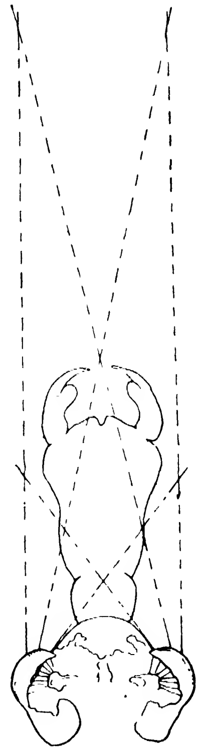


FIG. 111.—Distance perception. Head of *Aeschna* nymph with extended labium

The broken lines are the visual axes of selected ommatidia arrived at by observation of the pseudopupil. The points of intersection of these axes determine the position and distance of objects (*after* BALDUS).

SIMPLE EYES

Stemmata—The eyes of larval and pupal forms are termed stemmata or lateral ocelli. These are very variable in structure. In larvae of Lepidoptera, Trichoptera, *Sialis*, *Myrmeleon*, &c., they form a group, each member of which has a structure something like a single ommatidium of a compound eye (Fig. 112). The eyes of Collembola are of the same kind; there are about eight on either side, each with the structure of an ommatidium of eucone type. On the other hand, in larvae of Tenthredinidae, many Coleoptera, &c., each eye, of which there is usually only one on either side, consists of a single transparent lens-like thickening of the cuticle (the cornea) with underlying epidermis, and below this a number of retinulae each composed of two or three innervated visual cells grouped round a rhabdom (Fig. 113). The visual cells may themselves contain pigment; or there may be pigment cells of variable distribution. In some larvae the eyes are rudimentary; for example, in *Ceratopogon* they consist only of a pair of visual cells and two overlying pigment cells.[53]

There is no doubt that in all cases the stemmata are organs for the perception of light; and in some insects, in spite of their very simple structure, they also subserve colour vision and a rudimentary perception of form. An appreciation of colour is seen in caterpillars of *Vanessa* and *Pieris*, which are attracted by the green colour of leaves or pieces of paper, irrespective of the colour of the surroundings, but more so on a white than a black background. This attraction to green disappears at pupation, when brown and black are preferred.[35]

An appreciation of form is shown by *Lymantria* larvae which will walk towards vertical silhouettes, such as cylinders of brown paper against a light background.[60] When such pillars are of equal width, the newly hatched larvae of this species prefer the taller; pillars of 9 and 10 cm. can be differentiated with certainty at a distance of 30 cm., those of 10 cm. being chosen. It can be

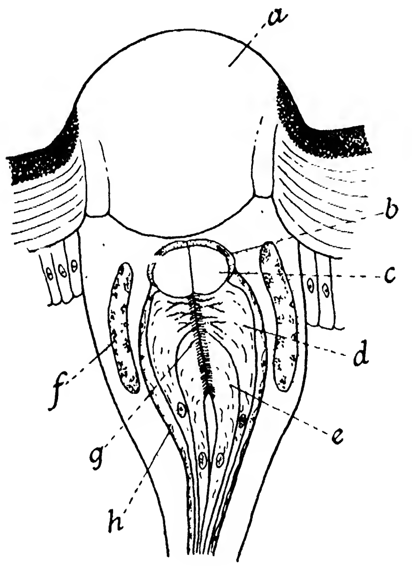


FIG. 112.—Simple eye, type i. Stemma of larva of *Gastropacha rubi* (*after* DEMOLL)

a, cornea; **b**, cells of crystalline body; **c**, crystalline body; **d**, distal sense cell; **e**, proximal sense cell; **f**, giant or mantle cell; **g**, rhabdom; **h**, pavement epithelium.

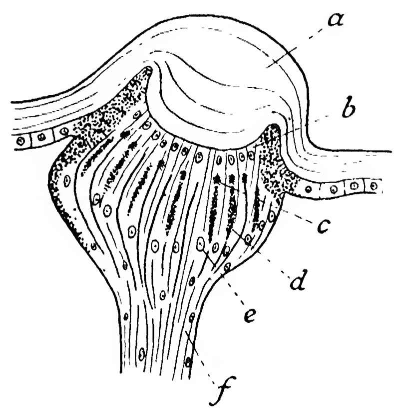


FIG. 113.—Simple eye, type ii. Ocellus of *Aphrophora spumaria* (*from* IMMS *after* LINK)

a, lens; **b**, matrix cells of lens; **c**, rhabdom; **d**, pigment; **e**, nuclei of retinula cells; **f**, nerve.

shown that objects of the same outline which subtend the same angle appear of equal size to the larvae. When different colours are compared blue appears the most attractive.[47]

In larvae of *Cicindela* there are six stemmata on each side, two large, two small and two vestigial. In the large type there are up to 6,350 visual cells, and the single lens is quite efficient. Therefore, although a clear perception of images is not possible, they can certainly form visual patterns of some detail. In the capture of prey by these larvae the axis of direction of each stemma is very important. The prey is perceived (though only if it is moving) at a distance of 3–6 cm.; the larva snaps at it only when it comes within reach of the mandibles; in the perception of distance all the stemmata clearly function together as a unit.[29]

In Muscid larvae there is a pair of conical sense organs remotely resembling ocelli in structure though devoid of pigment, at the tip of each of the bilobed maxillae. These are thought by some to be the light-perceiving organs which are certainly located in this region. Thus some authors claim that the responses to light disappear when the tips of these maxillary lobes are removed [24]; others state that the sensitivity to light persists after their destruction [74, 85]; and since this sensitivity increases with age it has been suggested that the imaginal discs of the adult compound eyes are the sensitive organs.[74]

Ocelli, or dorsal ocelli, are the simple eyes of adult insects which occur, usually on the frontal region of the head, alongside the compound eyes. Their structure varies, but in general it agrees with that of the second type of stemma described above, in which there is a group of visual cells below a common lens. Where the ocelli are best developed, the lens and retina are well differentiated; but in some cases the ocellus may be nothing more than an unpigmented spot in the cuticle with a few irregularly arranged rhabdoms beneath.

There can be no doubt that the ocelli are light-sensitive organs. The ocelli of Agrionids and Libellulids have a tapetum, and when brought from the dark into a bright light they show a rapid movement of pigment which spreads in a few seconds like a brown curtain across the white fundus.[59, 44] Although the relation does not always hold,[89] in many insects the ocelli seem designed to admit much more light than the compound eyes. The ocelli of *Eristalis* are estimated to have an aperture of f 1·8, in females of *Formica* f 1·5; whereas the facets of the compound eyes have an aperture of f 2·5–4·5 and will therefore have only $\frac{1}{16}$ of the luminosity of the ocelli. On the other hand, the superposition eyes of Sphingids and Noctuids are more luminous than ocelli, and in these forms ocelli are absent.[45] But the ocelli seem very ill designed for the perception of images; for not only is the angular separation of the rhabdoms very great (it varies from 3°–10° in various insects),[45] but the image is usually focussed far behind the retina [89]: in *Eristalis* the retina lies 0·11 mm. from the lens; the focal plane lies at 0·29 mm.[45]

From these considerations of structure it has been concluded that the ocelli are adapted to the immediate perception of small changes in light intensity.[44, 45] But although they must be stimulated by light the insect shows no outward response to such stimulation. Ants with their ocelli alone uncovered behave as though blind [45]; bees [69] and *Drosophila* [10] after blackening the compound eyes no longer show any reactions to light. But although in these insects the ocelli by themselves are incapable of evoking reflex movements in response to light, they have the effect of accentuating the responses of the insect to light stimuli received through the compound eyes. In both *Drosophila* [10] and *Apis* [69] the insect with ocelli uncovered responds more rapidly to changes in light intensity. The ocelli are therefore regarded as 'stimulatory organs' which increase photokinesis (p. 157). But their function will not necessarily prove to be the same in all insects.[69]

Dermal light sense—In some insects the general body surface seems to be sensitive to light. Blind cavernicolous beetles of the genus *Anophthalmus* respond to the light of a candle.[67] Blinded *Blatella* [36] and *Periplaneta* [11] still settle for preference in the dark after complete blinding. Caterpillars will go towards a source of light after the eyes are blackened.[59] And the bug *Neides* in a state of partial hypnosis turns over when the eyes are blackened much more

readily in the light than in the dark, as though activated by a dermal light sense.[34] Many caterpillars which feed in exposed situations and are coloured like their surroundings, place themselves so that the light always falls upon them from a particular angle. In this response the light receptors appear to lie in the general body surface.[80a] In the case of *Chironomus* larvae the responses to light after blackening the eyes are due to light entering the translucent head capsule and reaching the eyes from below.[70] But this cannot be a general explanation of the results, for *Tenebrio* larvae avoid the light even after decapitation.[83]

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Chapter VII

Sense Organs : Mechanical and Chemical Senses

MECHANICAL SENSES

THE SIMPLEST mechanical sense is that of touch. This may be subserved by (i) receptors of contact projecting from the surface of the body, (ii) sense organs stimulated by the strains set up in the cuticle by pressure, or (iii) organs sensitive to changes of tension within the body. These three types of receptors merge rather indefinitely into one another : in the last analysis they are all stimulated by pressure changes in the end organ of the nerve. Further, any of them may serve to detect either stimuli from the outside world, or stimuli resulting from the position or movements of the insect's own body ; that is, they may act as exteroceptors or as proprioceptors. Proprioceptive stimuli may result from the action of gravity ; so that the same receptors may subserve a static sense. Finally, the perceptions of these same sense organs may be so refined that they respond to the pressure changes and movements in the air, which we perceive as sounds ; that is, they serve as organs of hearing.

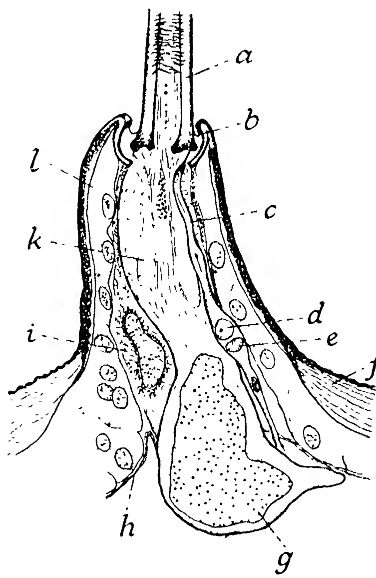

FIG. 114.—Section through base of tactile bristle in larva of *Vanessa urticae* (*after* HSÜ)

a, base of hair ; *b*, articular membrane ; *c*, scolopoid body ; *d*, sense cell ; *e*, neurilemma cell ; *f*, cuticle ; *g*, trichogen cell ; *h*, basement membrane ; *i*, tormogen cell ; *k*, vacuoles in trichogen cell ; *l*, epidermis.

Tactile spines and hairs—Nearly all the spines and hairs of the body surface are sensory end organs. Each individual organ was termed by Haeckel a ' sensillum ' ; as we shall see later, these sensilla may assume many forms and serve many different senses. The simplest are the widely spread articulated setae (Fig. 114). As a rule, each of these is supplied by a single bipolar sense cell, invested by neurilemma, situated beneath the epidermis and sending its distal process to end in the margin of the socket, its proximal process to the central ganglion. The distal process (the ' terminal filament ') is often deeply staining, and at moulting it is shed with the cuticle ; it is sometimes stated to be a chitinized product of the sense cell.[96]

Sensory hairs of this type occur all over the body. They appear to be stimulated by movement of the spine in its socket. Thus if the electrical

changes in the crural nerve of the cockroach are recorded while the hairs on the tibia are deflected with a fine glass needle, two types of response can be obtained. (i) In the case of the finer hairs sudden deflection causes a short burst of impulses which is over in a fraction of a second (that resembles the response of the touch receptors in vertebrates). (ii) In the case of the stout spines adaptation is much slower; the response to the same angular deflection varies according to its direction, being maximal towards the proximal end of the segment; and the initial frequency of discharge depends on the speed of deflection. (In these characters it resembles more closely the response from the proprioceptors of vertebrates.[80])

These articulated hairs are clearly tactile receptors. They are very numerous at the tip of the tibia and on the tarsal segments, where they evidently perceive contact stimuli. They occur all over the cerci of crickets and other Orthoptera in which they perceive, among other stimuli, earth-borne vibrations when they rest on the ground, and air currents when they are held aloft.[81] The antennae of some insects are richly supplied with such hairs and are then obviously tactile organs: this is particularly so in cave-dwelling insects such as crickets, in the cockroach [14] or in the earwig. When *Forficula* moves along it palpates in every direction with its antennae; if one is removed it palpates all sides with the remaining antenna.[118] The antennae of *Rhodnius* have a limited number of such spines, which cause instant withdrawal of the antennae if they are touched; they stand out in all directions in such a way as to prevent any object from coming in contact with the more delicate sensilla with which the antennae are richly supplied (Fig. 115).[119] In *Gerris* they are numerous on the trochanter and femur where these rest on the water; here they enable the insect to maintain its normal position on the water surface and serve to perceive vibrations in the surface film set up by other insects on which they prey.[113] Along the margin of the wing in Lepidoptera there is a fringe of sensory hairs of the same type, and some of the wing scales, particularly on the marginal veins and the lower surface of the wing, are innervated: these have been regarded as responding to air movements during flight [36, 105]; they probably respond, also, to contact stimuli during the folding of the wings.

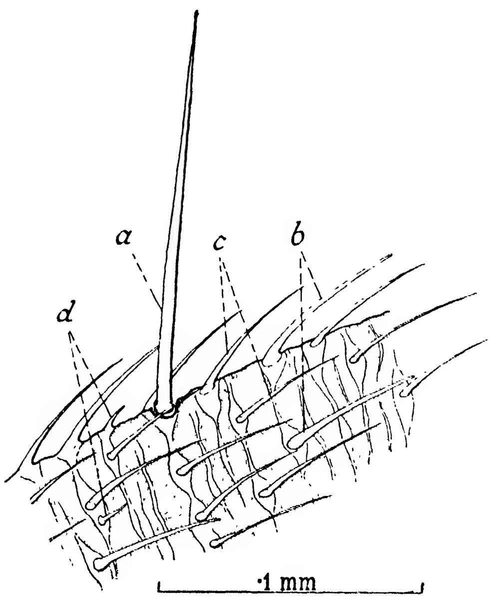

FIG. 115.—Surface view of part of an antennal segment in *Rhodnius* (*after* WIGGLESWORTH and GILLETT)

a, tactile spine; **b**, thin-walled sensilla, probably olfactory (*cf.* Fig. 132); **c**, fine thick-walled sensilla, perhaps temperature receptor (*cf.* Fig. 137); **d**, short sensilla showing the same structure as **c**.

Where very numerous and delicate hair-like sensilla occur on the antennae, it has been suggested that they serve to detect the changes in air currents when

the insect in flight approaches some solid object, and so enable it to avoid collisions in the dark by the same mechanism as is employed by bats.[79] The importance of air movements is seen also in flies. As is well known, flies are instantly disturbed by a sudden movement of the hand in their vicinity; they are not so disturbed if they are protected by a glass container.[40]

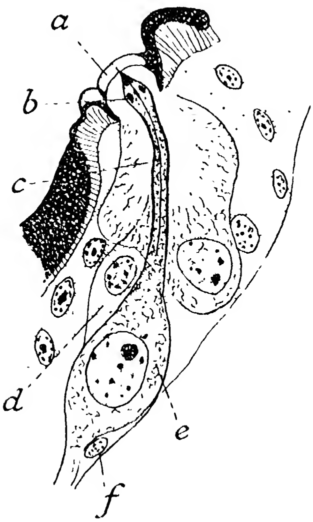

FIG. 116.—Campaniform sensillum on the cercus of *Gryllus domesticus* (*after* HSÜ)

a, dome-shaped plaque; b, scolopale; c, vacuole in membrane-forming cell; d, accessory cell; e, sense cell; f, neurilemma cell.

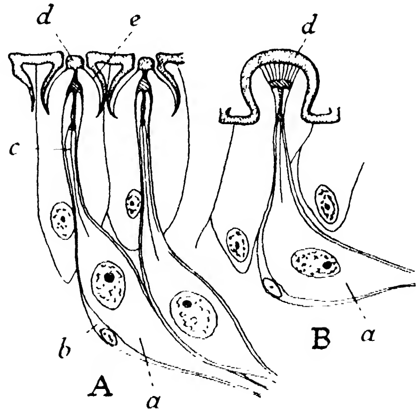

FIG. 117.—Campaniform or Hicks' papillae from halteres of Diptera (*from* DEMOLL *after* PFLUGSTAEDT)

A, from *Calliphora*, cut transversely to the long axis of the papilla; B, from *Sarcophaga*, cut in the long axis of the papilla. a, sense cell; b, neurilemma; c, terminal filament running to scolopale; d, longitudinal thickening of the dome; e, thin membrane forming side of dome.

Campaniform sensilla—In the campaniform sensilla no outgrowth from the cuticle remains. The terminal filament from the sense cell ends in a 'sense rod' or 'scolopale' which is inserted into a dome-shaped area of relatively thin cuticle (Fig. 116). This dome is usually overhung by the more rigid cuticle around. Sometimes it is circular and symmetrical; sometimes it is elliptical; and sometimes, notably in the so-called 'Hicks' papillae' on the halteres of Diptera, in which the dome is elliptical, the surface is thickened in the longitudinal axis so as to form a stiffening rod that has been compared with the crest of a helmet (Fig. 117).[22, 76] They are similar in the legs of *Periplaneta* (Fig. 118).[77]

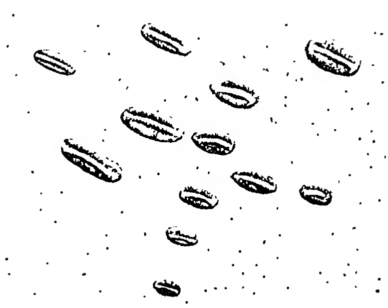

FIG. 118.—Group of campaniform sensilla on hind leg of *Periplaneta*, showing the longitudinal thickening of the dome (*after a photomicrograph by* PRINGLE)

These organs occur in all parts of the body. In *Dytiscus* they occur particularly on the basal joints of the extremities and head appendages.[54] They are regularly distributed on the wings of Lepidoptera and other insects.[105] In the altered form described above they are present in great numbers on the halteres of Diptera.[76] They are very numerous on the gills of *Caenis* nymphs (Ephemeroptera).[26]

Almost all authors are agreed in regarding the campaniform sensilla as being sensitive to mechanical stimuli. They have been thought to respond to water pressure in aquatic insects, as in the antennae of *Dytiscus* [54] or the gills of the Ephemerid *Caenis* (in which they may be stimulated perhaps by vibrations in the water or by the impact of suspended particles).[26] It has often been suggested that the organs on the wings and elsewhere may be sensitive to changes in air pressure during flight, and thus serve to correlate such changes with the wing vibrations—as in *Dytiscus*,[54] in the bee,[73] and in Lepidoptera.[36, 93] They are much reduced in number on the wings of *Calandra granaria* which has lost the power of flight, as compared with *C. oryzae* which still flies [87]; but they persist in female Lepidoptera with vestigial wings and in Pupipara where the wings are reduced in both sexes, and in ants they are confined to the region proximal to the preformed line of cleavage.[11]

Alternatively they have been thought to respond to bending of the cuticle. Where they are radially symmetrical they will react to flexion equally in all directions; where they are elliptical, as in Hicks' papillae, they will be affected only by bending in their long axis, i.e. in the direction of the stiffening rod. The side walls of the dome are so thin and delicate that they will not influence the movement of this rod to which the scolopale is attached; whereas compression in the long axis will exert tension on the sensory ending (Fig. 117).[22] This conception is supported by the fact that in the halteres a single group of papillae all have their long axes disposed in the same direction, but different groups are set at an angle of approximately 90° with each other.[22] And on the legs of *Periplaneta* the organs occur in groups all elongated in the same direction (Fig. 118).[77] In the cerci of *Gryllus*, &c., it has been suggested that they may be stimulated by the bending of the cuticle set up by pressure upon adjacent spines.[96]

In *Periplaneta* there is a group of campaniform sensilla in each segment of the palp, each group being supplied by a large sensory fibre, formed apparently by fusion of the axons of the peripheral sense cells. When the segments of the palp are forcibly moved a regular synchronous electrical discharge is set up in this nerve; but the organs seem to be very little stimulated by active movements of the joints. Similar results are obtainable with the legs of *Periplaneta.* Each group of sensilla appears to act as a unit, and the evidence suggests that it probably responds to the compression component of the shear force. For example, in the tarsi the sensilla occur on the dorsal aspect of the segments; they will therefore be compressed when the foot is applied to any surface, and are perhaps responsible in part for the perception of contact stimuli.[77]

Chordotonal sensilla or scolopidia—These sensilla are generally believed to be derived from campaniform sensilla through their component parts becoming elongated and deeply sunk within the body.[12, 21, 22] In the usual type, the sensory rod and terminal filament, in which the distal process of the sense cell ends, lie in the axis of an elastic strand stretched between two points in the body wall (Fig. 119). One end at least is nearly always inserted into some pliable region of the cuticle, such as the intersegmental membranes.[28] They are exceedingly widespread in all parts of the body: in the legs, antennae (Fig. 120, *b*, *e*), palps, wing bases, &c., besides the general body cavity.[20, 28] They can be seen most readily in larvae of Nematocera, in which each segment except the first and last contains two pairs, a large and small. The respiratory

siphon of *Culex* larvae contains three pairs.[22] The larva of *Drosophila* has 90 chordotonal organs, attached at both ends to the epidermis.[51] They occur in the base of the wing in Lepidoptera [106] and at the base of the wing and halteres in Diptera.[51, 76]

In all cases there seems no doubt that the integumental scolopidia * are stimulated by changes in tension. Such changes may be induced by external pressure, or by the passive movements of the segments [22]; by the pressure or tension of the contracting muscles [51]; by general changes of internal pressure

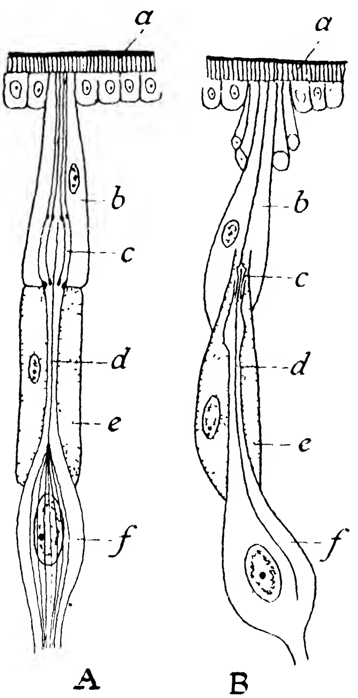

FIG. 119.—Scolopidia, schematic

A, from chordotonal organ (*from* DEBAUCHE *after* SNODGRASS) ; *B*, from Johnston's organ (*from* DEBAUCHE *after* EGGERS). *a*, cuticle ; *b*, distal or cap cell ; *c*, scolopale or sense rod ; *d*, terminal filament ; *e*, middle or sheath cell ; *f*, sense cell.

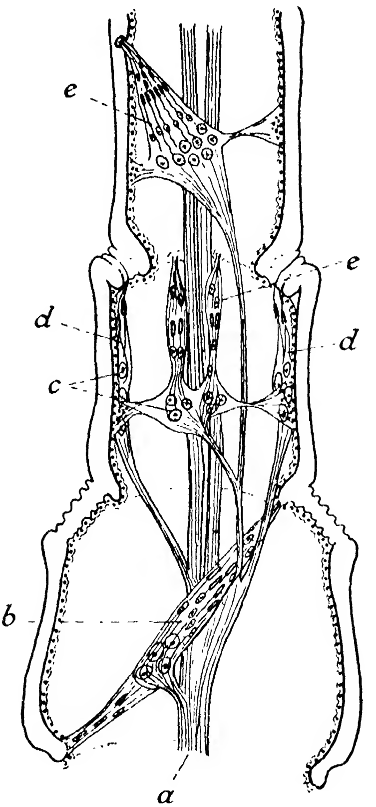

FIG. 120.—Arrangement of sense organs in the three basal segments of the antenna of *Meconema varium* (*after* DEBAUCHE)

a, antennal nerve ; *b*, chordotonal organ of scapus ; *c*, sense cells ; *d*, Johnston's organ ; *e*, chordotonal organ.

in the blood or perhaps in the tracheal system [49]; or, in some special cases, by sound vibrations (p. 141). They are not to be regarded as structures of constant function, but as nervous organs, peculiar to insects, which may be adapted to diverse physiological purposes.[21]

Johnston's organ—The second antennal segment or pedicellus always contains a group of sense organs very similar in structure to the chordotonal sensilla (Fig. 119, B ; 120, *d*). These are radially arranged, with their lower

* A special 'subintegumental type' will be mentioned later (p. 141).

insertion on the wall of the pedicellus and their upper insertion in the thin intersegmental membrane of the antenna. They are referred to as Johnston's organ.[28] The antenna, as we have seen, contains also a number of campaniform sensilla and independent chordotonal organs.[21] But in those insects in which Johnston's organ becomes more highly developed these begin to recede; and in Culicids and Chironomids (Fig. 121), in which it shows its highest degree of complexity, completely filling the great globular second segment, chordotonal organs and campaniform sensilla are entirely absent.[21]

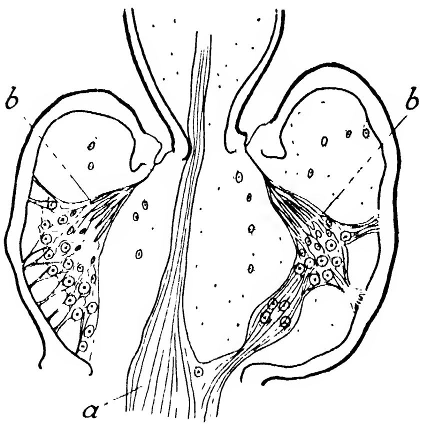

FIG. 121.—Longitudinal section of pedicellus of antenna in *Chironomus viridis* (*after* DEBAUCHE)

a, antennal nerve; *b*, Johnston's organ.

Judging from its structure, Johnston's organ must be stimulated by movements of the flagellum of the antenna on its base. It is therefore described in the bee, for example, as a 'statical organ' to register the flagellar movements.[62, 107] Such movements may be active movements induced by the muscles, or passive displacements due to contact or to air currents.[28, 112, 119] It is very well developed in the whirligig *Gyrinus*, and since the antenna is carried in the surface film as the beetle moves rapidly about, it has been suggested that the Johnston's organ enables it to perceive the curvature and finer vibrations of the water surface and so avoid collision. Beetles deprived of the antennae collide repeatedly with the sides of their aquarium.[30]

Proprioceptive organs—It is clear from the foregoing account that many different sense organs responding to mechanical stimuli may serve as proprioceptors—sense organs stimulated by the movements of the insect's own body or the strains set up in it by the tension of its muscles.

(i) The peripheral nerve net (p. 94) contains bipolar and multipolar sense cells which give out fine branches ending freely under the general body surface in soft-skinned larvae, as in caterpillars [55] and in *Melolontha* (Fig. 122),[123] or in the intersegmental membranes of forms with a rigid cuticle,[22] for example, in the articulations of the limbs in *Aeschna* nymphs.[123] These endings are believed to be stimulated by tension in the body wall or movements of the joints.[22]

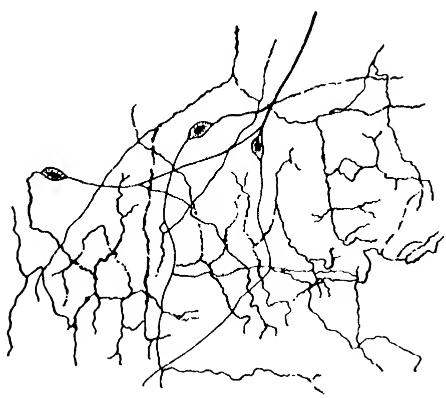

FIG. 122.—Subepithelial nerve plexus with bipolar sensory cells in the larva of *Melolontha* (*after* ZAWARZIN)

(ii) A similar nerve net is present in the peripheral part of the visceral nervous system in *Melolontha* and *Oryctes* larvae, where this supplies the muscles

the mid-gut and oesophagus. It contains two types of sensory ending : a) a network of varicose branches enveloping the individual muscle fibres in a sheath or muff, very similar to the sensory muscle endings of vertebrates, and (*b*) diffuse branching endings between the muscle fibres (Fig. 123).[75] Similar endings occur in the muscles of the stomach, hind gut, &c., but whether they exist in the skeletal muscles is not known.

(iii) In some insects erect tactile bristles serve to detect the degree of flexion at the joints.[22] When *Periplaneta* falls though the air the thoracic segments move against one another and flight begins ; this response is attributed to stimulation of the tactile hairs in this region.[25] Tactile hairs doubtless serve as proprioceptors during the cleaning of appendages, &c.[80]

(iv) Many of the chordotonal organs must serve to perceive the movements and internal strains in the body [20, 22, 82] ; for example, in the base of the wings and halteres [106, 115] ; in Johnston's organ, which must often provide the chief proprioceptor of the antennae [28] ; in larvae of Diptera, where the organs are pressed upon by the contracting muscles.[51] It is conceivable that some of the chordotonal organs act as ' manometers ' detecting pressure in the tracheal system or the blood.[49]

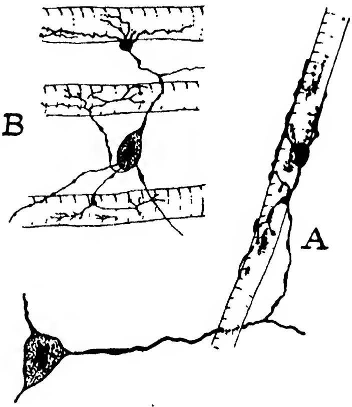

FIG. 123.—Two types of sensory nerve endings in the muscle fibres of the oesophagus of *Oryctes* larva

A, muff-like investment of the fibre ; *B*, fine diffuse nerve endings. (*After* ORLOV.)

(v) The slightest movement of the limb in *Periplaneta* causes an outburst of impulses in the nerve in the femur ; this proves the existence of tension receptors in the limb.[7] The campaniform sensilla are probably the chief organs concerned in this response.[77] Thus it would seem as though the insect responds chiefly to strains in the cuticle of the limb as a whole, whereas the vertebrate responds to tension changes in the individual muscles.[77] The campaniform sensilla in the wings are also regarded as proprioceptive organs.[11]

Organs of equilibrium—The stimulation of these various proprioceptive organs by the weight of the body acting upon the limbs must be an important factor in the equilibration of the insect in respect to gravity.[7] The sense of gravity is probably the sense of position and the sense of contact distributed over the whole body. Thus *Notonecta*, *Naucoris* and *Macrocorixa*, blinded and with weights attached to the body, are able to maintain their direction up and down in the water, and they can still do so after removal of the antennae and after section of the nerves to the abdomen.[74] *Dytiscus* tends always to climb upwards on an inclined slope (negative geotaxis). This response is not merely a mechanical effect of the weight of the abdomen, for it persists after the centre of gravity has been entirely changed by attaching a weight to the fore part of the body.[124]

In addition to this general sense, a few aquatic insects possess special static sense organs. The larva of *Limnophila fuscipennis* has a pair of sacs in the last abdominal segment, each with a sensory hair at its blind end and granules or statoconia in its lumen (Fig. 124). As this sac is rhythmically pumped up with

water and relaxed, the statoconia fall upon the sensory hair. The normal lar is positively geotactic; this response is abolished if the organ is removed.[1] In the larva of *Ptychoptera contaminata* there is a typical vesicular statocyst wit movable statoliths in the 10th and 11th segments of the abdomen.[113] And it has been suggested that the peculiar Graber's organ of Tabanid larvae is a static organ; but experiment does not appear to support this idea.[17]

Static organs of a totally different character occur in some aquatic Hemiptera. They consist of localized collections of tactile hairs placed at interfaces where the air continuous with that in the tracheal system comes in contact with the water. Any movement at this interface due to changes in pressure will be detected by the sensory hairs; and since the pressure of the water will be greatest in the most dependent parts, they will serve to orientate the insect in respect to gravity. Organs of this type are best developed in *Nepa*. In the nymphs the air is held in grooves, roofed over by long hairs, along each side of the ventral surface of the abdomen. On four of the segments there is a small interval

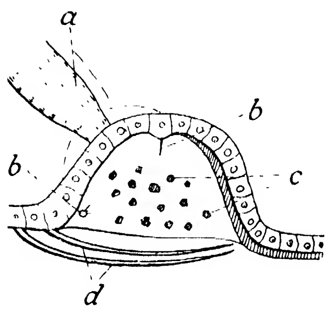

FIG. 124.—Static organ of Limnobiid larva *Ephelia*. Schematic section (*from* WEBER *after* WOLFF)

a, muscle; *b*, sensory hairs; *c*, statoliths; *d*, bristles closing the organ below.

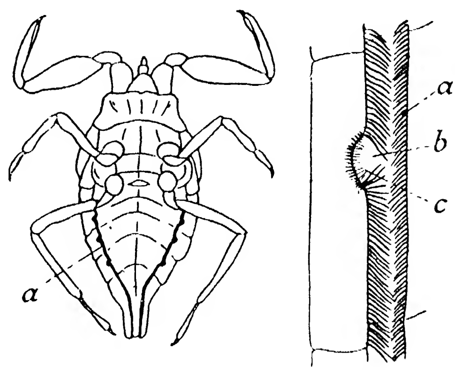

FIG. 125

A, nymph of *Nepa*, ventral view to show respiratory furrow, *a*. *B*, detail of part of respiratory furrow in nymph of *Ranatra* to show sensory pit (*after* BAUNACKE). *a*, respiratory furrow with roof of bristles; *b*, point of contact between air and water; *c*, sensory hairs.

where the long hairs are replaced by short sensory hairs, and it is here that the air comes in contact with the water (Fig. 125). In the adult a new sensory apparatus based on the same physical principle is developed in connection with three of the abdominal spiracles (Fig. 126). If a normal *Nepa* is blinded (for vision certainly assists in the orientation) and allowed to crawl on a small submerged seesaw towards the water surface, it turns round at once when the inclination is reversed; whereas the insect with the organs put out of action walks up or down indiscriminately.[10] In addition to these organs *Nepa* possesses a general sense of position resident in the appendages and persisting after section of the nerves to the abdomen.[74] In *Lethocerus* (Belostomatidae) the static organ consists of a pair of deeply sunk abdominal spiracles with overlying tactile hairs.[112]

The numerous proprioceptive organs on the wings (chordotonal and campaniform organs and tactile hairs) probably assist in the maintenance of equilibrium during flight. In Diptera this function seems to be centred particularly

in the halteres, which are well provided with such receptors. Some Diptera, such as Tabanids, are able to fly without the halteres [15]; but others, such as *Calliphora*, show complete loss of equilibrium in the air after their removal; they are able to fly only if equilibrium is restored by attaching a strand of cotton to the tip of the abdomen.[35] It is suggested that the mechanism by which the halteres serve as 'balancers' during flight is as follows. During the normal vertical vibration the obliquely placed rows of elongated campaniform organs at the base are not subjected to any shearing strain; but any lateral movement

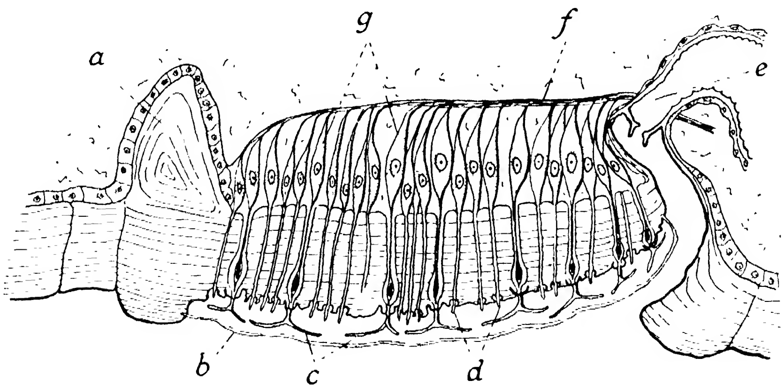

FIG. 126.—Transverse section through abdominal spiracle of *Nepa* adult showing the adjacent sense organ, apparently adapted to detect the hydrostatic pressure (*after* HAMILTON)

a, chitinous ridge around the organ; *b*, membrane covering the organ; *c*, flattened scale-like sense organs probably responding to pressure; *d*, peg-like sensilla; *e*, spiracle; *f*, nerve; *g*, bipolar sense cells.

of the haltere will stimulate them. It is supposed that the vibrating haltere with its terminal knob acts as an alternating gyroscope; consequently, if the insect is rotated out of the plane of their vibration lateral shearing forces will be set up in the cuticle of the base and these will be perceived by the campaniform sensilla.[35]

HEARING

The waves of air pressure which we recognize as sounds may be perceived by insects by two quite different mechanisms. The movements of the air which are secondary to the pressure changes may stimulate the more delicate tactile hairs, or special auditory organs (tympanal organs) may respond to the pressure waves themselves.[5]

Acoustic function of hair sensilla.—The perception of vibratory air movements is very widespread. Male mosquitos respond to such tones as will set the fine hairs of the antennae in motion.[68] Ants react to sounds so long as their antennae are intact, perhaps by the same means.[5] Caterpillars respond to sounds by cessation of movement and some contraction of the body; in *Vanessa antiopa* partial removal of the body hairs diminishes this response [69]; in *Datana* larvae the response fails when the hairs are loaded with water droplets or dry flour or when the body surface is anaesthetized with procaine.[1] Even relatively hairless forms such as *Danaus plexippus* are sensitive; perhaps very small hairs are concerned.[69] Hearing of this type has been demonstrated also in *Blatella germanica*, Ephemeroptera, *Crioceris* (Col.), &c.[6] And in insects

provided with tympanal organs (*Locusta*,[44] *Liogryllus*[85] and *Thamnotrizon*[86] (Orth.), *Agrotis* and *Catocala* (Lep.)[29]) it is probably responsible for the slight sensitivity to sounds which persists after these organs have been removed.

These vibrating hairs will respond to a wide range of tones. Caterpillars react at least from 32 vibrations per second to 1,024 vibrations.[69] Their properties have been studied in most detail in the anal cerci of *Gryllus* and *Periplaneta*, by amplifying the electrical responses in the sensory nerve and studying them by means of the cathode ray oscillograph. The nervous response to sounds observed in the cercal nerve is probably derived from the hairs on the cercus, since it is abolished if vaseline is applied to the ventral surface from which they arise, whereas vaseline applied to the upper surface bearing numerous campaniform sensilla has no effect. There seems to be no lower limit to the

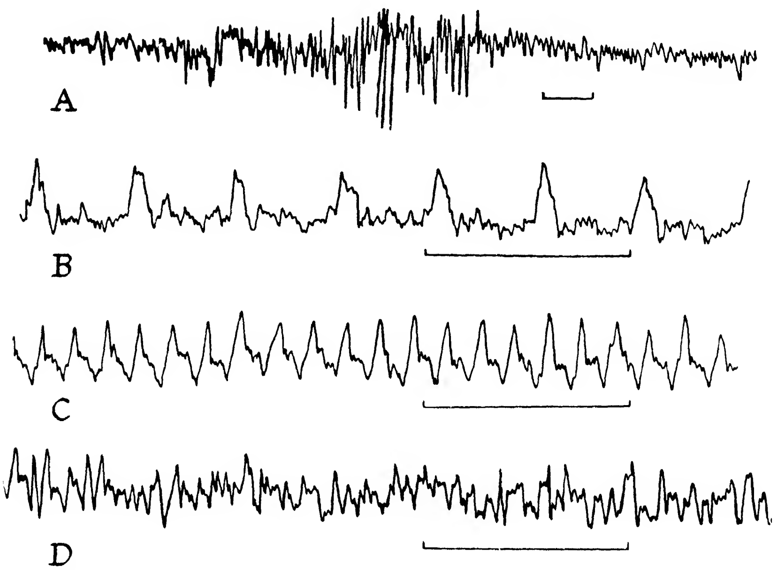

FIG. 127.—Electrical responses from cercal nerve of *Gryllus* (*from* PUMPHREY and RAWDON-SMITH)

A, response to a light puff of air; *B*, synchronous response to pure tone stimuli of frequency 100 cycles per second; *C*, synchronous response to pure tone stimuli of 300 cycles per second; *D*, irregular or asynchronous response to pure tone stimuli of 900 cycles per second. The straight line indicates 20 milliseconds in each case.

frequency which is effective; they will respond to low frequencies quite inaudible to the human ear. With pure tone sound stimuli from 50 up to 400 cycles per second the frequency of response is synchronous with the stimulus (Fig. 127, B, C). Occasional synchrony, due perhaps to the individual fibres responding to every other stimulus, may occur even up to 800 per second. Above this the response is asynchronous (Fig. 127, D); but it persists up to about 3,000 cycles per second or rather more.[81] It is possible, however, that the acoustic function of these organs is merely incidental; in the cricket the main function of the cercus is perhaps the detection of earth-borne vibrations; in the cockroach, in which the cercus is held erect, it may be a wind gauge.[81]

When the antennae are very light it is possible that the entire flagellum may vibrate in response to air-borne sounds, and then Johnston's organ may be an

auditory receptor. This has been suggested in the case of Culicids and Chironomids [28] and in *Reduvius personatus.*[112]

Tympanal organs—The chordotonal organs were so named by Graber [44] in the belief that they are always sensitive to sounds. That view is not supported at the present time. The bee, for example, which has numerous chordotonal organs, apparently cannot hear; it cannot be trained to associate sounds with the presence of food.[59] But all the evidence goes to show that where the chordotonal organs are associated with a cuticular drum or tympanum they do respond to sounds. The scolopidia in these organs may be of the type already described, with a filamentous insertion at both ends (integumental scolopidia) as in Acridiidae, Cicadidae, Lepidoptera, &c., or one of these attachments may be replaced by a large cap cell ending freely in the body cavity (' subintegumental scolopidia '), as in Gryllidae and Locustidae.

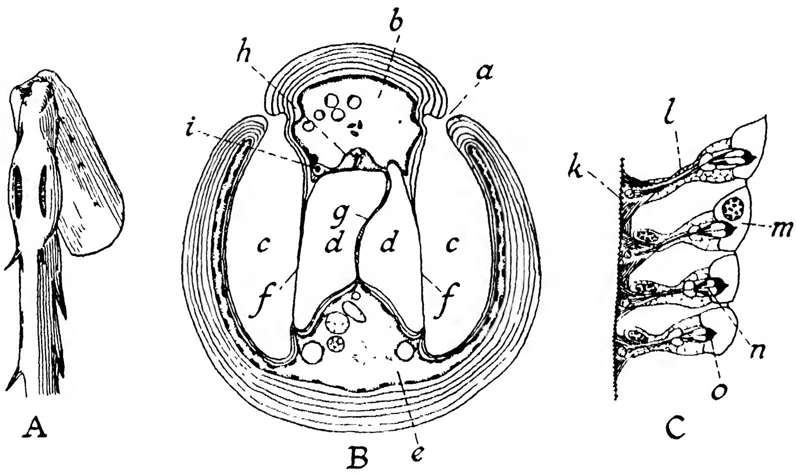

FIG. 128.—Tibial tympanal organ of Locustids

A, fore-leg of *Locusta* showing the slit-like openings of the tympanal sacs (*after* WEBER). B, transverse section of anterior tibia of *Decticus* at the level of the tympanal organ (*after* SCHWABE). a, cleft in anterior surface of tibia; b, blood channel; c, tympanal cavities; d, tracheae; e, blood channel with muscles, nerves, &c.; f, tympanum; g, rigid wall between the enlarged tracheae; h, crista acoustica with one chordotonal organ; i, sense cell. C, longitudinal section through a part of the crista showing four chordotonal organs (*after* SCHWABE). k, trachea; l, sheath cell; m, cap cell; n, axial filament; o, apical body.

All tympanic organs are fundamentally alike; a thin cuticular drum is exposed on one side to the external air, and a group of scolopidia is either directly connected to its inner surface or lies on a secondary membrane so situated as to move with it. In Acridiidae a pair of such organs occurs in the first abdominal segment; a large tracheal air sac covers most of the inner surface of the drum, and a group of integumental scolopidia is attached to the drum near one margin. In Locustidae a very complex organ is present near the base of each anterior tibia (Fig. 128). This part of the tibia is dilated, and has two fine slits directed forwards. These lead on each side into an invagination or pocket. Two tracheae separated by a rigid wall fill the space between the two pockets. Where each invagination is applied to the trachea its cuticle is very thin and forms the drum. No scolopidia are inserted into the drums themselves, but a row of subintegumental scolopidia is attached to the anterior surface of

one of the tracheae. These constitute the 'crista', the individual scolopidia of which become progressively shorter from above downwards (Fig. 128, C).[2, 95] In Gryllidae the structure is similar but the drums are not protected by an infolding of the cuticle.[22]

Rudimentary tympanic organs occur in the mesothorax of *Plea*, *Corixa*, &c. (Hem.), and in the mesothorax and metathorax of *Nepa* and *Naucoris*.[122] The chordotonal organs at the base of the wing in some butterflies, notably Satyridae, have their ventral insertion attached to a thin area of cuticle which constitutes a drum.[106] There are well-developed tympanal organs in the metathorax of many Lepidoptera (Notodontids, Lymantriids, Noctuids, &c.) (Fig. 129) and in the first abdominal segment of Geometrids and some other Lepidoptera.[29] A highly complex tympanal organ occurs in the second abdominal segment of *Cicada*; here the drum lies between the outer air and an abdominal air sac; the tension in the drum is regulated by a muscle; and about 1,500 slender scolopidia are present. In most of the tympanal organs it is obvious how the movements of the drum will stimulate the scolopidia; in *Cicada* it is uncertain whether the vibrating drum causes the organs to swing laterally, or to be

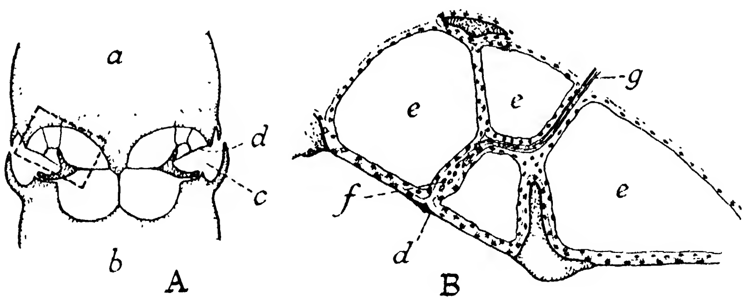

FIG. 129

A, horizontal section of thoracic tympanal organs of *Catocala*; *B*, detail of organ on the left side (*after* EGGERS). *a*, thorax; *b*, abdomen; *c*, tympanal pit; *d*, tympanum; *e*, tracheal air sacs; *f*, chordotonal organs; *g*, nerve.

extended and relaxed in their long axis, or whether vibrations are set up in the fluid parts of the organ and the scolopidia respond by resonance. Their length varies in the ratio of 2 : 3; it is therefore possible, if they act as resonators, that they might distinguish tones over about one octave.[108]

There is a good deal of evidence to prove that the tympanal organs are sensitive to sounds. Various Noctuid moths (*Catocala*, *Agrotis*, *Amphipyra*) will vibrate their wings or fly away in response to the high-pitched notes of a Galton whistle, under conditions where all but air conduction is excluded.[102] They do not respond if the tympanal organ at the base of the abdomen is pierced with a needle.[29] The same behaviour is shown by *Pyrausta* and *Larentia*, but never by Rhopalocera, Sphingids, Hepialids or other forms without drums.[29, 57] Whether the normal function of these organs in Lepidoptera is the perception of sounds, however, is open to doubt. Perhaps they respond to other air vibrations; for example, vibrations set up by the wings. They are poorly developed or absent in wingless females, and they are reduced in females of those Noctuids with abnormally large ovaries, in which wing reduction is only just beginning.[43] Possibly they are proprioceptive organs of some kind.[28]

Locustids of the genus *Thamnotrizon* normally chirp alternately with one another when their tympanal organs are intact ; but when these are removed they chirp irregularly. Here again the sound is air-borne ; for a pair of individuals placed in megaphones directed towards each other alternate properly ; they chirp irregularly if the megaphones are directed away from each other.[86] Females of *Gryllus* are attracted to the male by his chirping, from a distance of more than 30 feet, and males without their stridulating organs do not attract. Chirps transmitted by telephone will cause the female to go to the receiver; but this response is no longer shown if both tympanal organs are removed ; whereas removal of the antennae is without effect.[85] Similar evidence exists in the case of *Stenobothrus* and other Orthoptera.[6]

Auditory range of tympanal organs—The range of sensitivity of the tympanal organs has been investigated by observing the electrical response in the sensory nerves or central nervous system during stimulation by sounds of

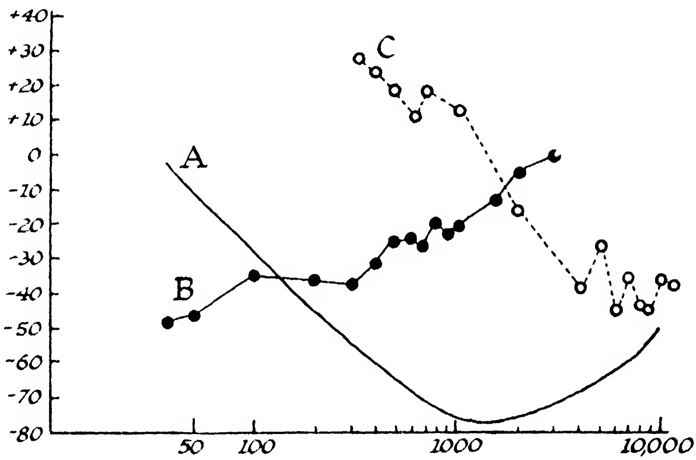

FIG. 130.—Auditory threshold at different frequencies

A, normal human threshold ; *B*, threshold of electrical response in cercal nerve of *Gryllus* ; *C*, threshold of electrical response in tympanal nerve of *Locusta*. Ordinate : sound pressures expressed as decibels above or below a zero approximating to 10 dynes per sq. cm. Abscissa : frequency in cycles per second on a logarithmic scale. (*After* PUMPHREY and RAWDON-SMITH.)

known frequency (Fig. 130). In an Acridiid, in which the response in the nervous system is found to disappear after destruction of the abdominal tympanal organ, the range extends from about 300 to 20,000 cycles per second. At low frequencies the tympanal organ is very insensitive as compared with the human ear : near the lower limit it requires for threshold stimulation a sound pressure more than 30,000 times that required by the human ear. But with increasing frequency the sensitivity increases, and at 10,000 cycles it is close to that of man. Sensitivity is greatest at this frequency, as contrasted with 1,500 in man.[117] In the Katydids *Ambycorypha* and *Pterophylla* a response was obtained over a range from 800 cycles up to 45,000 cycles, which is well above the human limit [117] ; in *Gryllus* it extended from 300 to 8,000 cycles.[117] In *Locusta* the sensitivity appeared to be still increasing above 10,000 cycles per second.[81]

Observations on the reflex responses of intact insects support these conclu-

sions. The Katydid *Thamnotrizon* will respond to frequencies from 435 to 25,000 per second,[86] *Acridium* from about 1,740 to 20,000.[4] In *Locusta* the type of nervous response is asynchronous at all frequencies; it is probable,[f] therefore, that these organs cannot distinguish tones. But being paired and, for sounds of low absolute intensities, very sensitive to intensity fluctuations, they are well suited to enable the insect to localize a source of sound.[81] The fact that females of *Liogryllus* are attracted to chirps transmitted through the telephone, in which the high frequencies will be lost, suggests that it is the rhythm of the sound rather than the frequency as such which is the essential stimulus for orientation.[117]

Sound production—Sounds may be produced by insects in many different ways.[56, 78] (i) By tapping, as in *Anobium*, which strikes its head against the woodwork where it burrows, or the soldiers of some termites, which strike the ground with their heads. (ii) By friction of the so-called stridulatory organs. In Acridiidae the upper surface of the costal margin of the hind wing is rubbed against the lower surface of the fore wing, or the inner aspect of each hind femur bearing a series of pegs is rubbed against the outer surface of the corresponding fore wing; in Locustidae and Gryllidae two modified areas of the fore wings are rubbed together; ants stridulate by rubbing a hard piece on the petiole up and down on a striated plaque at the anterior constriction of the abdomen [83]; some Coleoptera, Lepidoptera and Hemiptera have various other means of stridulating.[6] (iii) Vibration of the wings in flight (in *Bombus*, *Apis*, *Melolontha*, &c.) produces a note whose pitch is determined by the rate of vibration (p. 89). (iv) Males of Cicadidae have a pair of drums at the base of the abdomen; these are convex outwards and a powerful muscle is attached to the inner concave surface; when this drum is repeatedly drawn inwards and released it produces sound by the same mechanism as a rounded tin lid pressed inwards by the finger.[112] (v) High-pitched sounds may be produced by mechanisms which are not in all cases understood. In *Acherontia*, air appears to be forced out from the oesophageal diverticulum and sets a fold in the mouth cavity in vibration. In the queen bee they seem to be caused by air driven out of the tracheae.[113] This may also be the cause of the sounds produced by Calliphora; or these may perhaps arise from vibration of the surface of the thorax or perhaps friction of the wing bases against the schlerites.[56]

Many of these sounds are adventitious and have no physiological significance. Interest attaches chiefly to the stridulatory organs. In Orthoptera these are associated with the presence of auditory tympanal organs and, as we have seen, they are of undoubted importance in bringing the sexes together. But in beetles, Hemiptera, &c., it is very doubtful if the sounds are heard by other insects, and in ants it is doubtful if any sound is produced at all.[5] Stridulation in them seems to be a nervous reflex, the significance of which is unknown.

CHEMICAL SENSES

Nerve endings of many kinds are doubtless sensitive to irritant chemical substances. From this has been evolved the common chemical sense of primitive invertebrates. In insects and vertebrates this sense has become further differentiated into the two senses of taste and smell.[45] There is no absolutely satisfactory distinction between these two. For in insects both are subserved

by primary sense cells (p. 93); their distribution varies from one species to another, and they may be located in the same part of the body; although the sensitivity is usually far greater in the case of smell, as estimated by the lowest molecular concentration that can be detected, yet there are very great differences between species; for terrestrial organisms taste is a sense of solutes and smell a sense of vapours, but this does not apply to aquatic forms. Here we shall regard as taste the perception of the qualities of sweet, sour, salt and bitter, which are called tastes in man; other chemical perceptions will be included in the sense of smell.[39]

General chemical sense--Irritant substances such as ammonia or chlorine fumes may be perceived in many parts of the body. Probably they stimulate sensory endings which normally respond to other stimuli. The legs of termites are sensitive to such irritants [48]; the cerci of *Gryllus*, which show no true sense of smell, will react to essential oils,[96] as will also the basal segments of the antennae and many other parts of the body in *Tenebrio*.[103] It appears to be this general sensitivity which has given rise to the mistaken belief that the widespread campaniform sensilla are organs of smell.[38, 77]

Taste—Organs of taste are associated with the *mouth* in many insects. Collembola are repelled by bitter, alkaline, acid or salt tastes in the food [99]; bees reject honey treated with quinine or salt [120]; caterpillars make vigorous spitting movements of the mouth in response to salt, acid or bitter substances.[24, 27] In the bee sensory pits at the base of the tongue are perhaps the receptors [120]; in caterpillars the response is abolished if the epipharynx and hypopharynx are removed.[24] The oral lobes of the proboscis in *Calliphora* bear gustatory hairs along their margins.[47, 69] Hemiptera have a perforated area of cuticle in the pharynx with flask-like cells below; this is often regarded as an organ of taste, although no nerve supply to it has been demonstrated.[16]

After a course of training, in which the beetles are either rewarded with sweetened meat or punished with quinine-treated meat, *Dytiscus* can learn to associate a particular taste with the presence of food. After several months, for example, if accustomed to being fed after a salt taste, *Dytiscus* will disregard cotton wool containing sugar but respond to wool containing salt. In this way it can be shown that *Dytiscus* distinguishes the four taste qualities. Many of the receptors concerned are borne by the *palps*, but others (inside the mouth) remain after the palps have been removed.[9, 92] Similar results have been obtained with *Hydrophilus*; in this beetle sweet, salt and bitter tastes are perceived only with the apices of the maxillary palps, sour tastes with the tip of the labial palps, which are rich in rod-like sensilla.[88] The dung beetle *Geotrupes*,[111] the bark beetle *Rhagium* and the cricket *Liogryllus* [60] also detect sweet tastes by means of the palps.

In a few insects such as ants,[94] bees, and wasps, the *antennae* have been shown to bear organs of taste. The honey-bee and the wasps *Vespa* and *Polistes* can distinguish plain and sweetened water by means of the antennae, and this response to sugar is prevented if acid or quinine is added. The receptors are confined to the eight distal segments and are perhaps the pore-plates (*cf.* p. 150).[60, 69]

A more frequent site is the *tarsus* and distal end of the tibia. In the butterflies *Pyrameis*,[3, 69, 116] *Danaus*,[3] *Pieris*,[69] the flies *Drosophila*,[8] *Calliphora*,[19, 69] *Musca*,[23] and in the honey-bee *Apis*,[39] immediately the tarsi come in contact

with sweetened water the proboscis is extended (Fig. 131); this response is given by insects which pay no attention to water alone. The tarsi of *Pyrameis* are also very sensitive to quinine; $\frac{1}{32000}$ M quinine will prevent the response to sugar; but 4 per cent. sodium chloride is taken as readily as distilled water.[116]

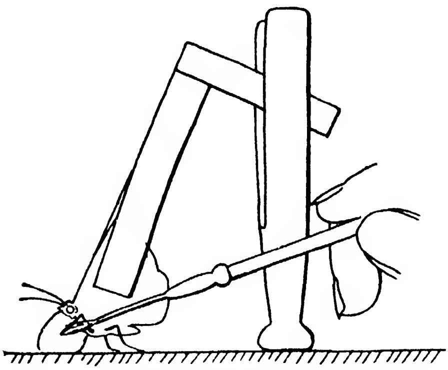

FIG. 131.—Demonstration of tarsal organs of taste in *Vanessa* (*after* MINNICH)

On touching the tarsus with a paint-brush moistened with sugar water the proboscis is immediately extended.

The tarsi of *Calliphora* are much less sensitive to acid and bitter repellant tastes than the tongue; they seem quite insensitive to salt.[47] In the case of *Pyrameis* the sensilla concerned consist of thin-walled tubular hairs in sockets, each associated with a large trichogen or glandular cell and a group of sense cells with a bundle of distal processes (*cf.* p. 150).[32]

Oral taste in the bee—The sense of taste has been studied in most detail in the mouth parts of the bee. Experiments in which the effects of training are combined with the repellant action of certain tastes have proved that sweet, bitter, acid and salt are recognized as separate qualities. As in the case of man, the sourness of solutions of hydrochloric acid is proportional to the hydrogen ion concentration, but weakly dissociated acids such as acetic are relatively more sour at a given *p*H. The bee is more sensitive to sodium chloride than man, but quinine is less repellant.[39]

Most substances which appear sweet to us are tasteless to the bee. For example, pentoses (arabinose, xylose, rhamnose), sugar alcohols (mannitol, dulcitol, sorbitol, erythritol) and many true hexose sugars (mono-, di- and trisaccharides) are all tasteless. Out of 34 sugars and related substances tested, 30 appear sweet to man, only 9 to the bee; all these nine being present in the natural food of the bee and capable of being metabolized by it (p. 285).[39] All artificial sweet substances, such as saccharin, are tasteless in dilute solution and repellant at higher concentrations; whereas acetylsaccharose, which appears very bitter to man, has no repellant effect on the bee (it has been proposed as a suitable substance to add to sugar so that this might be made unfit for human use and supplied free of duty to bee-keepers.[110])

The threshold of taste for cane sugar, as judged by the lowest concentration that is imbibed, varies from 1M to M/8 according to the richness and abundance of nectar in the field. The sensitivity falls with increasing age and the threshold is raised if a sweet mixture has been given shortly before the test; whereas in bees starved for some hours it will fall to M/16. The sweeter the mixture the more of it does the bee imbibe. For example, in one set of experiments bees took up 42 c.mm. of a M/2 sucrose solution, 49 c.mm. of 1M and 56 c.mm. of 2M. And the sweeter the mixture the more do the bees excite the other members of the hive on their return, by ‘round dances’ and extrusions of their scent glands.[39]

Oral taste in other insects—The sweetest sugar for the oral lobes of *Calliphora* is sucrose, with a threshold concentration of M/100–M/400;

glucose appears less sweet; lactose and maltose, with a threshold concentration of 128/100–64/100M less sweet still.[69] The threshold falls somewhat during fasting. These flies taste as sweet many more sugars than the bee, but they metabolize about an equal number. Some sugars, such as the pentoses arabinose and xylose, are not utilized although they taste sweet; others, such as galactose (which is not utilized by the bee), are metabolized by *Calliphora* although tasteless (p. 285).

Among ants there is much variation in the sense of taste. *Lasius*, *Formica* and the different species of *Myrmica* differ in the number of sugars that are apparently sweet, and in the relative sweetness of those sugars to which they are sensitive.[94]

Lymantria larvae, after being offered water until it is refused, will accept sucrose at a minimum concentration varying from M/2–M/10 and drink it vigorously. The M/10 solution may be accepted after water but refused after the M/2 solution.[24] Various caterpillars will ingest sodium chloride up to a maximum concentration of M/8, hydrochloric acid up to M/320 and quinine up to M/10,240; but these thresholds are raised if sucrose also is present.[27] Larvae of *Anosia plexippus*, which feed on milkweed, will commence eating other leaves if these are enclosed between slices of milkweed leaves, but almost at once they are rejected.[24] Presumably this indicates a perception of flavour not included in the four taste qualities. Similarly, larvae of *Pieris brassicae* and *P. rapae* attack any plants smeared with mustard oil, and even starch or paper so treated; the larvae of the saw-fly *Priophorus padi* feed on various Rosaceae, all containing the glucoside amygdalin; the beetle *Gastroidea viridula* feeding on *Rumex* appears to be attracted by oxalic acid and oxalates [104]; and the blood-sucking bug *Rhodnius* will not suck up warm serum through a membrane unless a little haemoglobin is added to it.

Tarsal and antennal organs of taste—Of all the gustatory organs those on the tarsus appear to be the most sensitive to sugars. They are tested by allowing the insect to drink distilled water until contact of the tarsus with water no longer causes the proboscis to be extended; in such insects sweetened water above a threshold concentration will still evoke a response. The level of this threshold depends on the state of nutrition. In *Pyrameis* fed regularly with sucrose the threshold for this sugar is around M/10–M/100, which agrees with the threshold of the human tongue (about M/50). But if sugar is withheld the threshold falls; after prolonged sucrose inanition it may be M/3,200, M/6,400 or even M/12,800 (200 times the sensitivity of the human tongue).[69] *Danaus* has been reported to give occasional responses to M/120,400.[3] This high sensitivity perhaps indicates that these tarsal organs serve to *call attention* to the presence of sweet substances rather than to test them as food.[39] In all cases sucrose is the sweetest sugar, as it is for the mouth parts; but sugars which appear sweet to the mouth parts may fail to cause any response by the tarsi; thus lactose, to which the oral lobes of *Calliphora* are very sensitive, has no effect on the tarsi even in 1M concentration.[69] The threshold for sucrose for the tarsal organs of the bee is given as 1M, and for the antennal organs M/12.[65]

Smell—The sense of smell in insects is located chiefly in the *antennae.* This was clearly established by Lefebvre (1838), who showed that the antennae of the bee are extended in any direction towards a needle dipped in ether; while in the absence of the antennae no response to odours is given; observa-

tions which have been confirmed on other insects (Ichneumonids, Sphegids, Muscids, beetles, &c.) by many authors.[34, 63] *Periplaneta* will locate cheese from a distance by means of the antennae, with which it reaches out in all directions as the source of smell is moved.[42] In termites the antennae alone are the organs for distinguishing true odours.[48] The louse *Haematopinus* extends its antennae towards a source of smell and with these intact can distinguish between the finger and a warmed rod.[114] The males of Saturniid moths, such as *Callosamia*, locate the female by smell, and this sense is confined to the highly branched antennae.[67] Females of *Habrobracon* discover their host *Ephestia* by smell, and the males find the females by the same means; if the antennae are amputated or covered with varnish these reactions fail.[71] Muscid flies find flowers or dung and the males locate the females by smell; accordingly the males and the dung-feeding species have antennae more plentifully supplied with sensory pits and sensilla.[61] The tsetse-fly *Glossina* can hunt and locate its host by sight alone, but the antennae are generally necessary to call forth the probing response after settling on the host.[72] *Scarabaeus* searching for dung holds up the antennae with the clubs spread open.[53] In *Tenebrio* the organs of smell lie especially in the last four antennal segments.[103]

But in many insects the *palps* also bear olfactory organs. At the apex of the palps of butterflies there is an olfactory pit well supplied with sensilla; and it is estimated that the sense of smell in *Pieris* is not reduced by much more than half if the antennae are amputated.[69] *Periplaneta* [42] and *Gryllus* [96] can locate food at close quarters by olfactory organs on the palps. *Geotrupes* has organs of smell on the maxillary and labial palps; but these are much less important than the antennae in finding dung at a distance.[111] The same applies to *Dytiscus*, except that here apparently the labial palps are not concerned: beetles trained to associate a given scent (coumarin, musk, &c.) with the presence of food can still respond so long as one maxillary palp remains intact.[92] While in *Hydrophilus*, where the antennae are specialized for purposes of respiration, the sense of smell appears to be confined to the palps; if these are removed, although the antennae are intact, the insect can no longer be trained to associate odours with the presence of food.[88]

Sense of smell in the honey-bee—The olfactory perceptions of the honey-bee have been studied in great detail by training foraging bees to associate particular scents, mostly flower scents and essential oils, with the presence of sugar.[38] By this means it has been shown that the bee can distinguish, for example, essence of orange from 43 other ethereal oils. There seems to be much in common between this sense in the bee and the human sense of smell. Thus the threshold concentration of these scents is of the same order as in man, though the bee seems better able to detect a given scent among a mixture of others. Some 32 odorous substances of no biological significance appear scented to the bee; whereas odourless flowers such as *Ribes rubrum*, *Vaccinium myrtillus*, &c., appear unscented; and substances such as nitrobenzol and oil or bitter almonds, or amylacetate and methyl heptenone which smell alike to man are also confused by the bee. Bees trained to particular scents lose the effect of training if the antennae are removed, whereas such treatment does not affect bees trained to colours. It is of interest, also, to note that while bees will learn to associate many ethereal scents with the presence of sugar, they cannot be trained in this way with foul-smelling substances such as skatol or asafoetida.[38]

Sense of smell in other insects—The sense of smell is of some importance in caterpillars. Larvae of *Anosia plexippus* separated by wire gauze from leaves of *Asclepias*, make searching movements as they crawl across these leaves, or come to rest over them, whereas they move straight ahead without stopping when passing over other leaves; and they refuse to feed on *Asclepias* leaves treated with scent or methyl alcohol.[24] The gregarious larvae of *Pieris brassicae* utilize the species odour, which they can detect at a distance of 5 cm., for collecting into colonies, and they will follow trails of this scent leading to such a colony.[121] Ovipositing Lepidoptera are presumably guided by smell in selecting their food plant: the Oleander hawk, *Daphnis nerii*, always chooses plants of the family Apocynaceae, *Nerium oleander* in the Mediterranean region, *Vinca major* in Northern Europe, *Trachelospermum jasminoides* in India; *Acherontia* chooses Solonaceae—potato or nightshade.[113] But the scents in question are not perceptible to man. Similarly, *Rhyssa* can recognize the larva of *Sirex* through several centimetres of solid wood. And males of Saturniid and Lasiocampid moths will assemble in large numbers around newly emerged females, attracted, apparently from great distances, by scents not detectable by man. Among ants, smell is enormously important both inside and outside the nest in a hundred different ways. Ants, like bees, distinguish by smell the members of their own community from others (ants of different genera which normally fight will feed amicably together after removal of the antennae [34]); they follow trails of scent to and from the nest; the sense of smell amounts in them almost to a sense of form and topography—if the trail followed by ants to and from a group of Aphids is reversed 180°, the ants are at once thrown into confusion.[34] The Hymenopterous egg parasite *Trichogramma* can detect (perhaps by the antennae, perhaps by the tarsal receptors) the odour imparted to eggs by another female which has walked over them, and in consequence avoids them for oviposition. If a host egg already contains a *Trichogramma* egg, this can also be detected, again perhaps by chemical sense, after the ovipositor has been inserted.[91]

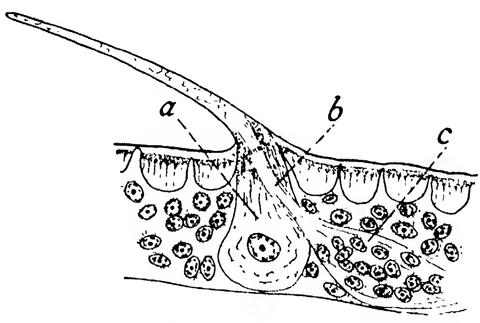

FIG. 132.—Section through the thin-walled type of sensillum (probably olfactory) on antenna of *Rhodnius* (*cf.* Fig. 115, *b*) (*after* WIGGLESWORTH and GILLETT)

a, trichogen or glandular cell; b, bundle of terminal filaments from the sense cells; c, fusiform mass of sense cells numbering about 15.

Chemo-receptive sensilla—All the sensilla of insects are covered by a continuous cuticular sheath [90]; but in certain of them the cuticle is excessively delicate, and it is natural to regard these as the chemoreceptors responsible for taste and smell.* The cuticular parts of these thin walled sensilla may be elongated in the form of slender hairs (sensilla trichodea olfactoria) as in the antennae of the wasp,[107] of *Rhodnius* (Fig. 132),[119] and of Saturniidae [79] or the tarsi of *Pyrameis*.[32] Or the hairs may be shortened to cones or pegs

* The campaniform sensilla have occasionally been regarded as olfactory organs; but their distribution does not agree with that of the sense of smell,[38] and in the cockroach they show no electrical response to olfactory stimuli.[77]

(sensilla styloconica and basiconica) as in the antennae of *Periplaneta*,[42] *Tenebrio*,[103] *Geotrupes*,[111] *Dytiscus*,[54] Hymenoptera,[107] and the apex of the palps of *Hydrophilus*.[88] Sometimes the thin-walled cones may arise from the floor of a single small pit in the cuticle (sensilla coeloconica) as in many dung beetles,[111] *Dytiscus*,[54] Lepidoptera,[13, 79, 93] &c. Sometimes a number of cones or rods arise together from the floor of a deep pit, as in the antennae of Muscids (Fig. 133)[61] or at the apex of the palps in Rhopalocera (Fig. 134).[84]

In Hymenoptera, thin-walled hairs of this type may become applied to the surface of the antenna. Sometimes such hairs are stiffened by a longitudinal thickening. And it has been possible to trace the evolution of these structures until the hair has disappeared and they consist of a flattened plate of fairly

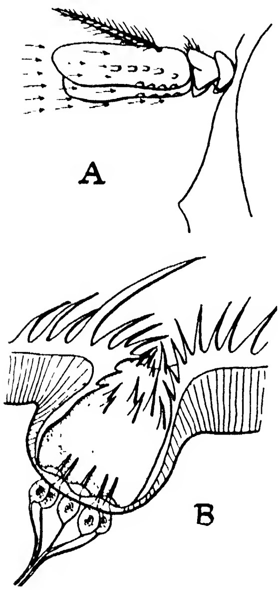

FIG. 133

A, antennae of Muscid fly erected during flight so as to expose the olfactory pits to the air stream ; B, detail of olfactory pit showing sensory rods each supplied by a single sense cell. (*After* LIEBERMANN.)

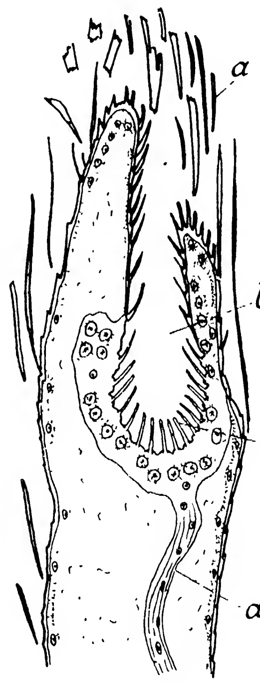

FIG. 134.—Longitudinal section of tip of labial palp of *Pieris rapae* showing pit containing olfactory rods (*after* HSÜ)

a, covering scales ; b, pit ; c, sensory rods ; d, nerve.

thick cuticle surrounded by a very delicate membrane (' pore-plates ' or sensilla placodea) (Fig. 135, *a*).[109] Such plates may be rounded, as in *Apis*,[107] *Melolontha*,[111] *Dytiscus*,[54] or so elongated that each will occupy the entire length of an antennal segment, as in wasps and many Ichneumonids.[107] The male honey-bee has about 30,000 of these organs on each antenna, the worker about 6,000.[107]

These thin-walled sensilla generally have no socket. The tormogen cell is often absent. But the trichogen cell is usually very large and secretes a clear fluid which fills the cavity of the hair or cone. The sense cells are generally multiple ; they may form a cluster of twenty or thirty, ensheathed in a nucleated coat continuous with the neurilemma of the nerve.[84] The proximal processes

of these cells form the afferent nerve ; the distal processes are united to form a bundle (the terminal filament), often with a group of little rod-like structures on its course, which penetrates the cavity of the sensillum to its tip in the case of the cones and hairs [98] or ends on the delicate marginal membrane in the case of the pore-plates.[107]

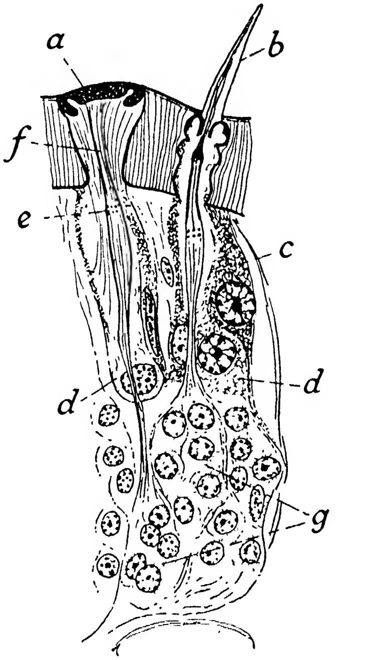

FIG. 135.—Sensilla on antenna of *Apis* (*after* HSÜ)

a, placoid sensillum ; *b*, thin-walled trichoid sensillum ; *c*, distal sheath cell ; *d*, basal sheath cell ; *e*, sensory rodlets ; *f*, terminal filament ; *g*, sense cells.

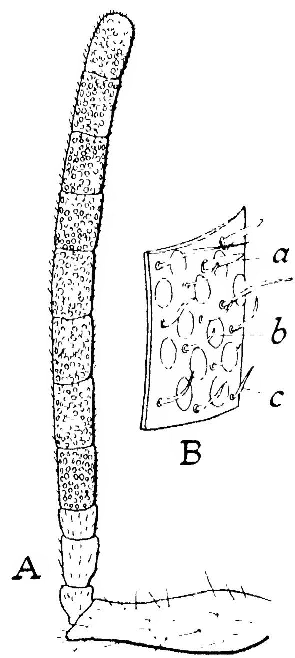

FIG. 136

A, antenna of honey-bee worker, medial surface, to show distribution of pore-plates (*after* v. FRISCH) ; *B*, detail of a part of one segment (*after* SNODGRASS). *a*, sensory peg ; *b*, pore-plate (*cf.* Fig. 135, *a*) ; *c*, thin-walled sensory hairs (*cf.* Fig. 135, *b*).

The evidence that the thin-walled sensilla are in fact the chemoreceptors is for the most part indirect. In Lepidoptera the sensilla styloconica and basiconica are most numerous on the antennae of the male.[13] Among Muscids the rods in the so-called olfactory pits average 820 in dung-feeding species, 494 in flower-haunting forms which orientate themselves in part by sight, and they are more numerous in the males than the females.[61] During flight these flies erect the antennae so as to expose these pits most effectively to the air (Fig. 133) : in *Drosophila* the sensitivity to odours is reduced if the antennae are fastened down—they will respond to a given scent at 5 cm., when normal insects with free antennae under the same conditions will respond at 22–35 cm.[33] The elongated pore-plates in *Habrobracon* are much more conspicuous in the male.[71] In *Dytiscus* the distribution of the organs of smell on the antennae and palps has been determined by eliminating the various segments ; this distribution agrees closely with that of the 'goblet-shaped organs' which approximate to the pore-plates of Hymenoptera.[92] In the honey-bee the pore-plates are confined to the eight terminal segments of the flagellum (Fig. 136) ;

a bee with only one of these segments remaining can still recognize the scent to which it has been trained and can be trained again to a new scent; whereas this power is lost if this one segment is removed. Bees in which all the sense organs except a group of pore-plates on one antennal segment have been extirpated still show slight olfactory responses.[38]

TEMPERATURE AND HUMIDITY

Temperature sense—All insects are sensitive to high temperatures and avoid places which are unduly hot. In grasshoppers this sensitivity is distributed over the entire body, but is most marked in the proximal half of the antennae and on the pulvilli and tarsi of the hind and fore legs.[41] *Liogryllus* perceives thermal stimuli chiefly with the antennae but also with cerci, wings, abdomen, &c.[50] The avoidance of high temperature by the bugs *Pyrrhocoris* and *Lygaeus* is greatly upset if the last segment alone of the antenna is removed.[50] The antennae of Phasmids are sensitive to excessively high temperatures [18]; and *Collembola* perceive warmth chiefly through the antennae.[99]

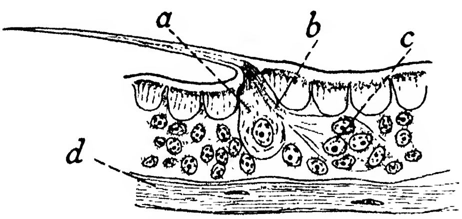

FIG. 137.—Sensillum on antenna of *Rhodnius* (*cf.* Fig. 115, *c*); perhaps temperature receptor (*after* WIGGLESWORTH and GILLETT)

a, trichogen or glandular cell; *b*, bundle of terminal filaments from sense cells; *c*, fusiform group of sense cells numbering about 5; *d*, nerve.

In some insects which suck the blood of mammals the perception of warmth by the antennae is an important factor in the location of the host. The mosquito *Anopheles* will attempt to probe warmed glass [64]; the lice *Pediculus* [37, 66] and *Haematopinus* [114] respond to warmth as well as to smell, and will follow a warm glass rod in all directions; the sense is distributed over the body but its chief site is the antenna. The blood-sucking bugs *Cimex*,[89, 97] *Triatoma*,[46] and *Rhodnius*,[119] and the flies *Stomoxys* and *Lyperosia* [58] are attracted to their host by warmth as well as smell and will probe warmed cotton wool or a tube of warm water.

In most of these insects it is doubtful whether there are any special sensory endings which are stimulated by high temperature; but in an insect such as *Rhodnius*, in which the antennae are exceedingly sensitive to very slight differences in the temperature of the air (*Cimex* orientates itself to a warm tube 1° C. above the surroundings from a distance of 1 cm.[97]), it is certain that specialized receptors must exist. The antennae of *Rhodnius*, besides the long tactile hairs, and the thin-walled trichoid sensilla which are presumably olfactory, bear a vast number of very fine but relatively thick-walled trichoid sensilla, each provided with a group of about 6 sense cells; these are probably the temperature receptors (Figs. 115, *c*; 137).[119]

Humidity sense—Some insects certainly choose their resting places according to the humidity (p. 159) but little is known of the sensory mechanism concerned. *Sminthurus* and other Collembola are very strongly 'hydrotactic'; they seek out moist places, perceiving not merely the humidity of the ground but of the air.[99] The earwig avoids humid resting places, the chief site of the moisture receptors being apparently the ventral surface of the abdomen.[118]

The honey-bee,[52] and the blood-sucking Muscids *Stomoxys* and *Lyperosia*,[58] are able to perceive water from a distance, perhaps by means of special receptors in the antennae. The mosquito *Culex fatigans* avoids high humidities above 95 per cent. relative humidity. Over this part of the humidity scale it is sensitive to differences of 1 per cent. R.H.; whereas between 30 and 85 per cent. R.H., humidity differences of 40 per cent. cause no response.[101] Whether the receptors concerned are responding to physical or chemical stimulation is not known.

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Chapter VIII

Behaviour

THE PHYSIOLOGICAL study of behaviour consists in the analysis of the movements of the whole organism into a series of reflexes or observed correlations between stimulus and response. Theoretically, in a reflex, the response is a function of the stimulus. But, as we saw in Chapter V, the relation between the two may be affected by a great variety of third variables—the number of times the stimulus has been given, the changes of adaptation or fatigue in the synapses, or the preceding activities of the nervous system, as in the reflex chain. In the behaviour of intact organisms such third variables lead to much apparent inconsistency in the relation between stimulus and response. In practice, therefore, the analysis of behaviour along these lines does not go very far, and in many descriptions of behaviour psychological conceptions, particularly conation, the innate seeking of some end or goal, are introduced in order to supplement the physiological mechanisms.

KINESIS AND RELATED PHENOMENA

Stimulatory organs—In addition to receiving specific stimuli, the majority of sense organs, when they are stimulated, have the effect of increasing the reflex excitability of the nervous system. Their action seems to be necessary in order to open the nervous paths, allow reflexes to take place, and so enable the nerve centres to carry out their normal function. Sense organs having this effect are spoken of as 'stimulatory organs' [117]; and the increased activity due to their action is termed 'kinesis'.

The *compound eyes*, for example, have an important stimulatory function. In *Periplaneta*, exposure to light greatly reduces the threshold of response to other stimuli.[11] The rate of walking in *Popillia japonica* is accelerated as the light intensity is increased.[74] The moth *Larentia truncata* responds to sounds by flying away when it is on a pale background, but it cannot be shifted by sounds when on a dark background.[56] And many day-flying butterflies (such as *Erebia* species) are entirely incapable of flight in the absence of bright sunlight.[7] If the eyes of *Macroglossa* are darkened it settles at once, folds the wings and lays back the antennae; if it is flying in a room lit with electric light, it immediately falls to the ground if the light is switched off.[41, 59]

The *ocelli* of adult insects which, as we have seen (p. 127) evoke by themselves no visual responses, are perhaps specific stimulatory organs which increase the reflex reactions to stimuli received by the compound eyes, and thus enable the insect to respond to changes in light intensity at a level of general intensity at which they are normally incapable of movement (Fig. 138). In *Drosophila* suddenly exposed to the light, movements begin in an average

of 4 seconds; if the ocelli are blackened the latent period is prolonged to 5 seconds; and after cutting off the light, activity diminishes more rapidly in the insect with ocelli intact.[7] Similarly, bees wake up very quickly in the light with functional ocelli, very slowly if the ocelli are covered. If they are exposed to two lights of different intensities bees normally go to the brighter; but if the median ocellus and one lateral ocellus are occluded, they always go to the side with the sound ocellus, even if the light on this side is considerably weaker, as though the optic centre of this side were more strongly stimulated.[75]

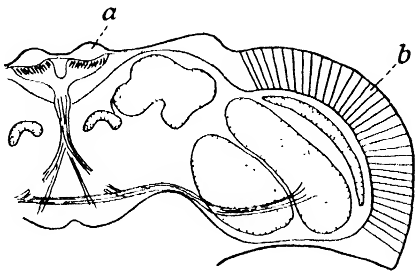

FIG. 138.—Transverse section of head of *Drosophila* showing the close association of the nerve tracts of the compound eyes and the ocelli (*after* BOZLER)
a, ocelli; *b*, compound eyes.

An example of the *antennae* serving as 'stimulatory organs' is afforded by the blood-sucking bug *Rhodnius*. If both antennae are removed, *Rhodnius* settles into a state of sleep or 'akinesis' from which it is aroused with difficulty, not only by such stimuli as air currents (which are normally perceived by the tactile spines and perhaps the Johnston's organ of the antenna), but by vibrations or violent stimuli to the abdomen. The antennae clearly have a kinetic function and normally keep the nervous system in a state of 'tone' in which it will respond readily to stimuli of all kinds. Air movements probably provide the chief stimuli concerned in this function.[115]

In addition to their function as organs of equilibrium (p. 138) the *halteres* of Diptera have been regarded as important stimulatory organs. It is supposed that the chordotonal and campaniform sensilla which they carry are stimulated by their vibratory movements, and that these stimuli facilitate the conduction of the reflexes concerned in flight.[16, 17] Suspended flies continue flying for a much shorter time after each stimulus if the halteres are removed[26]; in *Tipula*, both wings and legs are weakened by removal of the halteres, and *Sarcophaga* deprived of halteres can scarcely flutter the wings.[16] In Strepsiptera the fore wings have become similarly transformed into club-shaped halteres richly supplied with campaniform sensilla (Fig. 139), and they appear to have the same function: if they are amputated the insect loses the power of flight and finally the hind wings just stay at rest.[104] The vibrations of the tarsi of the middle pair of legs in the dung beetle *Sisyphus* before and probably

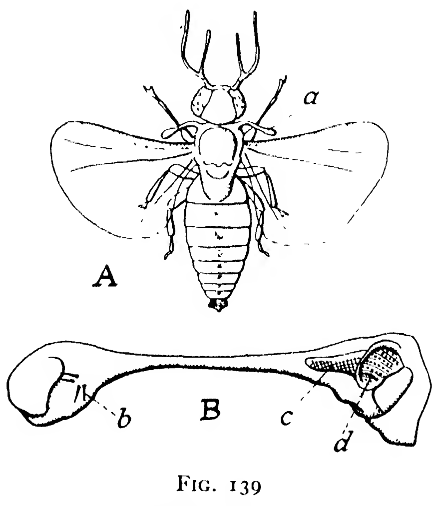

FIG. 139

A, male of *Elenchinus* (Strepsiptera) showing the fore wings (*a*) reduced to halteres (*after* ULRICH). *B*, dorsal view of left haltere of *Calliphora* (Dipt.) to show the distribution of sense organs (*after* PFLUGSTAEDT). *b*, hair sensilla on the terminal knob; *c*, area of campaniform sensilla (Hicks' papillae); *d*, area of insertion of chordontonal organs.

during flight has been regarded as serving a stimulatory function [64]; as have also the vibrations of the wings of many insects, and the vigorous pumping movements made by *Melolontha* before flight.[17] Many Diptera, such as *Tabanus* [17] and *Calliphora* (if it is suitably balanced and allowed a day or so to recover from the operation) [27] can still fly without reduction in the frequency of wing beat after the removal of the halteres; their stimulatory function, therefore, is not always necessary.[27]

Phototonus—The Asilid fly *Proctacanthus* becomes immobile or makes feeble incoordinated movements if the eyes are blackened. In the dark or with blackened eyes its legs collapse; in the light it raises its body well above the ground. The light appears to exert a general effect on muscle tonus. If one eye alone is blackened, tonus is exaggerated on the uncovered side; and if the upper or lower parts of both eyes are covered, different groups of muscles are affected so that the insect takes up characteristic attitudes (Fig. 140).[33] The same response is shown by the aquatic bug *Ranatra* [43] and the Syrphid *Eristalis*.[71] It is sometimes termed ' phototonus ', being regarded as a stimu-

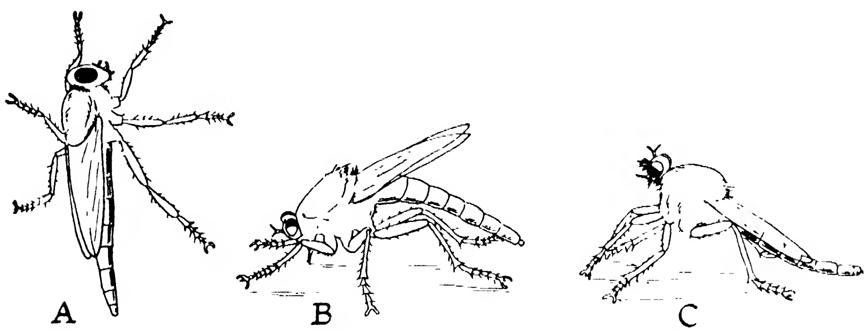

FIG. 140.—Attitudes taken up by the Asilid *Proctacanthus* with different parts of the eye blackened (*after* GARREY)

A, right eye blackened; fly viewed from above; B, upper halves of eyes blackened; C, lower halves of eyes blackened.

latory effect of different regions of the eye upon different groups of muscles.[33, 117] But, as we shall see, this type of behaviour is susceptible of other explanations (p 164).

Hygrokinesis—Little is known of the mechanism of the response of insects to differences in humidity; but in an insect like *Forficula*, which will not settle in contact with moist surfaces, the effect of moisture appears to be stimulatory or kinetic.[112] And the same applies to *Locusta migratoria* which, when offered two alternative humidities, always shows a preference for the drier at all parts of the humidity range, although dry air is not necessarily optimal for development or breeding. For when tested in an actograph, designed to give an automatic record of spontaneous movements, *Locusta* always shows most activity in moist air.[55] The insect will therefore come to rest more frequently in dry surroundings.

Stereokinesis or thigmotaxis—In all the stimulatory effects so far considered, the stimuli received in the sense organs have served to increase the excitability of the nervous system. But in the case of the mechanical senses the reverse effect is often seen: movements and reflex sensitivity are inhibited by stimulations. When the earwig comes to rest it brings as many of its tactile

receptors as possible into contact with some object; it may then require very strong stimuli to induce it to move. Although light ordinarily has a kinetic effect, the inhibitory influence of these contact stimuli may successfully compete with this, so that the insect remains at rest even when the eyes are brightly illuminated (Fig. 141).[112] This inhibitory effect of contact is termed 'stereokinesis' or 'thigmotaxis'. It is seen, also, in *Cimex* which, like the earwig, may settle in the light if its contact receptors are sufficiently stimulated[82]; and in the moth *Amphipyra*, which normally creeps into dark crevices, but will come to rest equally between glass plates.[65] Many insects, e.g. *Haematopinus*,[111] remain constantly in motion on smooth glass, but soon come to rest ('sleep' or 'akinesis') on a rough surface.

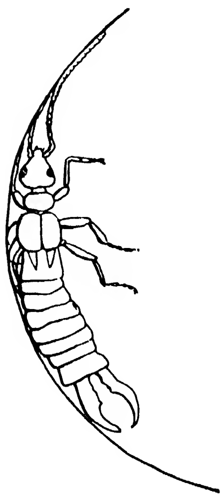

FIG. 141.—Example of thigmotaxis. Position taken up by *Forficula* in a circular glass container (*after* WEYRAUCH)

The inhibitory effect of contact is seen very strikingly in flying insects. Loss of contact between the tarsi and the ground, in Odonata[103] and in Muscid flies and other insects,[26] immediately induces flight. A suspended fly with vibrating wings instantly comes to rest if a little ball of cotton-wool is placed between the feet[26]; in *Musca*, contact with a single claw of one leg is sufficient to inhibit flight, and this inhibition disappears after amputation of the tarsi.[21] If any part of the body of a suspended insect is touched, it will extend the legs to this point, secure contact, and so inhibit the flight movements.[42] Similarly, the swimming movements of *Aeschna* nymphs are reflexly inhibited if a paper ball is placed between the feet[100]; and the turning over of beetles or caterpillars which have fallen on their backs can be prevented if they are given some object to hold.[17]

Reflex immobilization, hypnosis, &c.—Akinesis induced by contact stimuli is the normal state of rest or sleep in insects. But in response to various mechanical stimuli the nervous system may be thrown into a state of abnormal inhibition. This state is sometimes compared with the local fatigue or inhibition which occurs in a reflex when the stimulus has been repeated a number of times; but instead of being localized it spreads throughout the nervous system.[113] It is termed 'hypnosis' or 'reflex immobilization'; but it is not always clearly distinguishable from stereokinesis or immobilization through passive contact stimuli.[41] It is sometimes regarded as a reflex with a precise peripheral localization, the receptors being constant in a given species[80]; but it can often be induced by a great variety of stimuli—repeated pressure, vibration, sudden seizure. In the earwig it is most readily induced by holding the pincers in forceps and drawing the insect along on the surface of cloth or paper[113]; in *Ranatra* it can be brought on by touching, rolling between the fingers, &c.[44] In many insects, beetles, bugs, caterpillars, it may be induced by sudden loss of contact between the feet and the ground, as when the plant on which the insect is walking is suddenly jarred; it is then commonly termed 'thanatosis' or 'death feigning'.[6, 34] In *Triatoma* the state may supervene naturally during feeding.[39] In *Dixippus* it shows a diurnal rhythm and comes on during daylight; in *Gerridae* it may occur naturally

or can be induced by pressure on the leg [42]; in these insects it appears to be a protective adaptation to life among plants.[93] The rolling or 'spiral reflex' of some caterpillars, and the 'warning attitudes' assumed by other caterpillars and saw-fly larvae, seem to be special cases of the same response.[66, 96]

During this condition of immobilization, all reflexes for locomotion and the correction of posture are inhibited, sensation is reduced, and the muscles are in a state of tonic contraction.[41] In *Ranatra* [44] and *Dixippus* [88] the condition shows a striking resemblance to the state of 'catalepsy' in man. There is the same insensibility to stimuli, the same absence of fatigue, and the muscles show the same characteristic '*flexibilitas cerea*'—they are plastic and yielding so that the limbs retain any bizarre position into which they are forced (Fig. 142).

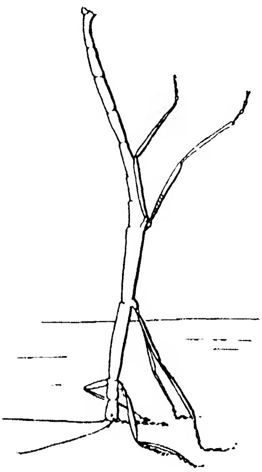

FIG. 142.—Catalepsy in *Dixippus*. The appendages have been forced into appropriate positions and the insect balanced on its head (*after* SCHMIDT)

If *Dixippus* is cut through between the meso- and metathorax while it is immobilized, the posterior fragment quickly recovers its activity; it becomes excessively responsive to reflex stimulation, and cannot be immobilized again; while the anterior fragment remains in its cataleptic state.[88] Similarly, if *Ranatra* is divided between the 1st and 2nd thoracic ganglia, both separated fragments will show the response, but it is of much shorter duration in the posterior fragment; insects without the head can be immobilized only for brief periods.[44] Thus the chief nervous centre concerned seems to lie in the brain.[93] If the response is induced repeatedly by appropriate stimulation it persists a shorter and shorter time until finally the nervous system becomes too excitable and the insect is quite refractory.[44, 60]

ORIENTATION

A great part of behaviour consists in orientation—the direction of the movements of the organism in space in response to external stimuli. For the purposes of physiological description such responses are regarded as being made up of a series of 'forced movements' or 'taxes', that is, reactions which invariably follow a given stimulus when the organism is in a given physiological state. Such 'forced movements' are themselves made up of a succession of reflex responses. But in dealing with whole organisms, in which the central mechanism of the response is quite obscure, it is often convenient to classify the mode of orientation without attempting to press the analysis to the level of these component reflexes.

Mechanisms of orientation—The various suggested mechanisms of orientation have been brought together into a single comprehensive theory by Kühn.[25, 62] It will be convenient to set out this classification here, although it must be realized that several of these mechanisms may be acting at one and the same time, and that there are orientated movements which do not fall naturally into the scheme.

In the first place the insect may reach its destination without being truly

orientated. This may occur in two ways. (i) Certain stimuli have a *kinetic effect* upon the animal and cause it to move at random until, by chance, it enters a region where such stimuli are wanting and there comes to rest. Such responses were considered in the last section. (ii) The animal, moving at random, may encounter adverse stimuli; whereupon it shows a reflex 'avoiding reaction' and, according to whether the avoiding reaction is the result of a stimulus above or below the optimal intensity, it is eventually forced to move away from or towards the source of stimulation. This mechanism is termed *phobotaxis* or the 'trial and error' response.

Responses during which the animal is truly orientated are termed *topotaxis*; and these again fall into two main groups.

(i) *Tropotaxis.* This mechanism is synonymous with the 'tonus hypothesis' advocated particularly by Loeb [65] under the name of the 'tropism theory'.* It implies that the muscular tone on the two sides of the body is proportional to the intensity of stimulus received in bilaterally symmetrical sense organs, and, this being the case, the animal when it moves turns towards the side on which the muscular action is strongest. It continues to turn until the sense organs on the two sides are equally stimulated. Muscle tone is then equal also and the insect moves in a straight line towards or away from the source of stimulus.

(ii) *Telotaxis.* This includes all those responses in which the organism 'fixates' one source of stimulus with its sense organs and advances towards it, or orientates itself in respect to it, in such a way that a certain region of the receptor apparatus is always acted upon by the chosen stimulus, other sources of stimulus being disregarded. The mechanism is defined as a series of reflexes, evoked and ended by the localization of the stimulus on the sensory surface.

Theoretically, these types of orientation may be effected through any of the senses. Thus response to light may be 'kinetic', 'phobotactic', 'tropotactic', &c.; response to smell may be a 'chemophobotaxis', a 'chemotropotaxis' and so forth.

Orientation by light—The kinetic effects of light have already been discussed; in addition to these, orientated movements to sources of light play a large part in the behaviour of insects. In the simplest cases these movements are responses to differences in light intensity perceived by the light sensitive organs. Where the eyes are more complex, the localization of the source of stimulus on the retina becomes important. And this leads on to examples in which the insect is orientated by the visual pattern.

Some insects are photopositive; they move towards the source of light; others are photonegative. Some, such as Muscid larvae, will always show the same response. In others it appears only when they are in a given physiological state. Young caterpillars of *Porthesia* (*Euproctis*) are strongly positive to light before they have fed; this response normally leads them upwards to the leaves of their food plant; but it is lost almost completely after feeding.[65] And at the time of the nuptial flight male and female ants are strongly positive, but this ceases as soon as they have shed their wings.[65] In the tsetse-fly *Glossina* and to a less extent *Stomoxys*, the response is affected by temperature.

* The word 'tropism' has been used in so many different senses that it is best avoided.[70]

These flies are usually positive, and go to the light half of a cage; but if the temperature is raised towards 40° C. the response is reversed and they make for the dark half, even though the temperature there be so high that they soon drop dead.[50] In many insects the response to light occurs only if they are 'alarmed' by some external stimulus: flies in a room are usually indifferent to light, but make for the window if disturbed; mosquito larvae show an immediate negative phototaxis if their vessel is jarred.[45] In such cases the response is sometimes regarded as an 'escape reaction' enabling the insect to flee straight to the light or to the dark in circumstances of danger.[28]

Phototaxis in insects with simple eyes—The larvae of Muscid flies show a well-marked negative phototaxis. The light receptors are confined to the oral region; their identity is not decided (p. 127), but it is certain that no kind of image formation is possible; the response is dependent solely on the perception of general light intensity. Exposed to a single light, the larvae move directly away from it. Exposed to two lights they move away along the resultant line dividing the angle between the two sources. If the two lights are of unequal intensity this line deviates away from the stronger; and although the degree of deviation is not exactly in accordance with the Weber-Fechner law (which lays down that the effective difference between two stimuli is determined by the percentage difference between them regardless of their absolute intensities), yet all the results obtained in such experiments can be explained by supposing that there is an effect on the musculature of the two sides of the body which is proportional to the strength of stimulus received by their sense organs.[79] This insect therefore affords the best example of 'tropotaxis', the insect behaves as though it were being forced by the effect on muscle tone to turn into such a position that the symmetrical organs receive the same illumination[65]; but it does not, of course, necessarily follow that such a mechanism actually exists.[79]

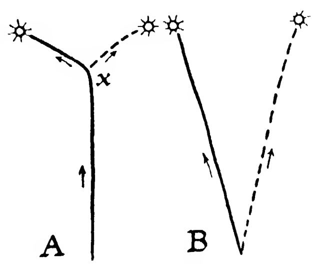

FIG. 143

A, *B*, two types of course followed by a positively phototactic caterpillar in the presence of two sources of light. *x*, deciding point ('Entscheidungspunkt'). (*After* LUDWIG.)

Young caterpillars of *Euproctis*, *Orgyia*, *Lymantria*, &c., before their first meal are positively phototactic; they will crawl straight towards a single source of light. If they are offered two sources of light they generally approach a variable distance along the resultant between them and then, usually when they lie at an angle of about 115° to the direction of the light, they begin to make searching movements and go straight to one light or the other (Fig. 143).[10, 63] This response is sometimes described as a 'tropotaxis' while they advance along the resultant, and a 'teletaxis' when they begin to move towards one only of the two lights. But the apparent neglect of the one source of light in the later stages may result from its falling outside the field of vision during the searching movements. The same response occurs, and the larva may go to either light, if the eyes are covered on one side.[10, 63] Some individual caterpillars when offered two lights will go straight to one of them. This again appears like a telotaxis or orientation towards a chosen source of stimulus. But it has been proved to depend on a natural bias of these individuals to incline in one direction as they advance.[35, 68] Larvae of *Ephestia*

are negatively phototactic; but they show this response to light stimuli only when they are in a definite state of stimulation. They then move directly away from a single light source and along the resultant if two lights are present.[8]

Phototaxis in insects with compound eyes—In insects with compound eyes capable of perceiving accurately the direction of a source of light, the mechanism of orientation becomes more complex. In a number of insects, such as the flies *Proctacanthus* [33] and *Eristalis*,[71] in *Ranatra*,[43] and nymphs of *Agrion* and *Cloëon*,[1] if the eyes on one side are blackened, the muscular tone on the uncovered side is relatively increased, so that the insect at rest inclines towards that side. This has already been quoted as an example of the stimulatory effect of light, or 'phototonus' (p. 159). Sometimes this effect becomes apparent only when muscular movements are made; the muscles on the uncovered side are then more active, and the insect moves in a circle in that direction.[1, 33] According to this explanation the orientation of these insects

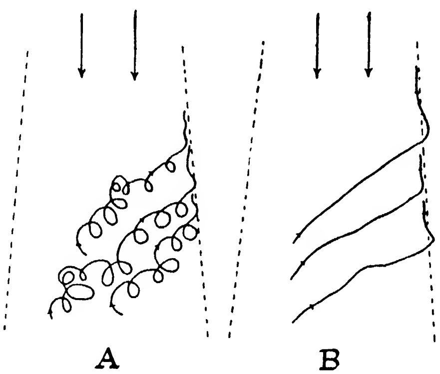

FIG. 144.—Tracks followed by *Dineutes assimilis* (Col.) with left eye blackened, in a divergent beam

Arrows show direction of light; broken lines show margin of beam. *A*, with eye dark-adapted; circus movements to the margin of beam, then straight to the source. *B*, after being in the beam some minutes (eye light-adapted); elimination of circus movements. (*After* CLARK.)

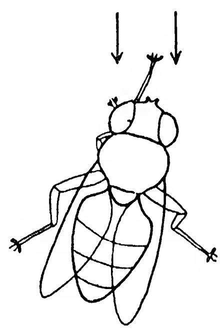

FIG. 145.—The fly *Eristalis* advancing towards a source of light after removal of the fore and hind leg on the right side (*after* MAST)

to light would be an example of 'tropotaxis'; the insect would be pictured as being orientated through the 'kinetic' effect of the sense organs being unequally distributed on the two sides of the body, a view which would unite 'kinetic' and 'orientating' responses into a single mechanism.[65] As the exposed eye becomes light-adapted, the exaggeration of tonus on that side is diminished; and in the insect at a given state of light-adaptation, the effect is dependent on the intensity of illumination: the greater the illumination the smaller the circle in which the moving insect turns.[33]

After a time, however, in such an insect with one eye blackened, the circus movements cease; the insect will go straight to the light, or turn in either direction (Fig. 144). Orientation cannot, therefore, be wholly dependent on the relative intensity of light received in the two eyes. Moreover, insects with one or more legs removed from one side can still walk straight to the light (Fig. 145). Orientation, therefore, is not necessarily dependent on balanced action of the locomotor appendages. These observations have been

made on *Vanessa antiopa* (Lep.),[22] *Eristalis* and *Proctacanthus* (Dipt.),[71] *Notonecta* (Hem.),[20, 67] *Dineutes* (Col.) [20] and *Apis* (Hym.).[105] They are best explained by supposing that illumination of the eye brings about a series of co-ordinated reflexes in the legs of both sides of the body specifically related to the localization of the stimulus in the retina.[71, 72]

According to this view, the eye consists of two functionally distinct regions: a large lateral zone which causes turning movements towards its own side, and a narrow medial zone which causes movements towards the opposite side. The boundary between these two constitutes the line of 'fixation', corresponding with the *area centralis* in the vertebrate eye; it evokes no turning movements (Fig. 146).[67] In the unilaterally blinded insect, in the light-adapted condition, the sensitivity of the lateral region is so great (in *Eristalis tenax* the central part of the eye is often more than 50 times as sensitive as the

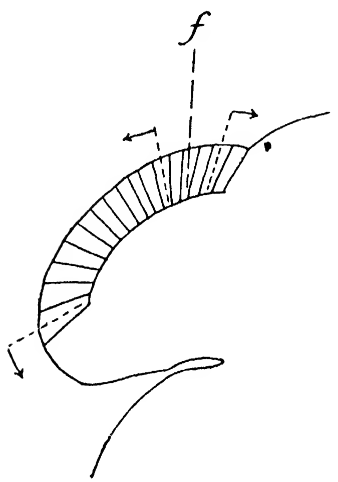

FIG. 146.—Diagram of the telotactic turning response in the compound eye (*from* LÜDTKE *after* KÜHN)

Arrows show direction of turning by induced illumination of different regions of eye. *f*, line of fixation.

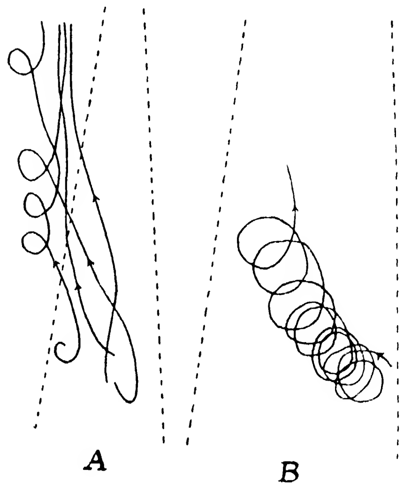

FIG. 147.—Effect of light reflected from the background on circus movements in *Notonecta* (*after* LÜDTKE)

A, tracks followed by four insects in dark-adapted condition in a beam of light, with a black background; B, track followed by similar insects in the presence of a white background.

anterior part,[23] in *Notonecta* the eye 78° behind the median axis may be 11 times more sensitive than 12° in front [67]) that the insect exposed to a beam of light turns repeatedly in circles towards the uncovered side. When the eye is in this sensitive state the light reflected from the background also contributes to the turning effect; turning is less marked if the background is black, with a white background it is continuous (Fig. 147).[20] Or if one eye is dark-adapted and the other light-adapted the same turning to the dark-adapted side is seen.[20] But as the eye becomes dark-adapted, and the sensitivity of the lateral region decreases, the insect is able to 'fixate' the source of light and go straight to it.[20, 71, 72] It is this response which is termed 'telotaxis'; it is the limiting condition between two sets of reflex turning movements.[67]

On this view the tilted positions taken up by flies after blackening parts of the eye, represent attempts to turn towards the light and bring it into the

axis of fixation without moving the feet. These positions are maintained after the light has been turned off; the light itself is therefore not necessary for the maintenance of the tonus effect.[71] The axis of fixation in these insects is not morphologically established; it varies with the physiological state of adaptation, &c.[67]; insects with the legs removed from one side move obliquely towards the light, fixating it with a new part of the retina. It is to explain adjustments of this type in the mechanism of orientation that psychological conceptions such as the 'attainment of the end in view' are sometimes invoked.[72]

'Light-compass' orientation—Insects may sometimes orientate themselves obliquely or at right angles to a source of light in such a way that one side is more strongly illuminated than the other. This behaviour was first observed in ants, which have been found in nature to maintain a straight

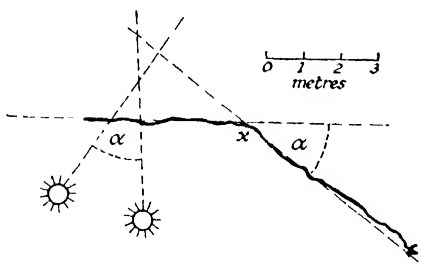

FIG. 148.—Course followed by an ant, *Lasius niger*

On reaching *x* at 2.39 p.m. it was placed in a box until 5.09 p.m. It was then released and followed a course making the same angle α with the original course as the sun had moved through in this time (*after* BRUN).

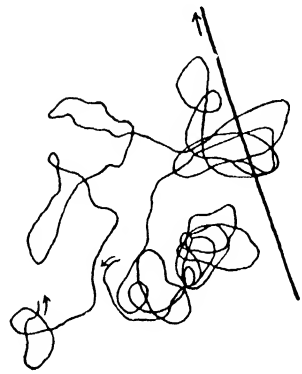

FIG. 149

The straight line shows the course followed by a Chrysomelid beetle exposed to a bright light. The convoluted line shows the course followed in the dark (*after* v. BUDDENBROCK).

course by moving at a fixed angle to the sun's rays (p. 173); if ants are detained on one of their excursions for an hour or two and then liberated, they follow a route which makes an angle with their outgoing route exactly equal to the angle that the sun has moved round (Fig. 148).[13] This behaviour was therefore termed 'light-compass' orientation.[85] But it has since been observed in many other insects under experimental conditions.

Larvae of *Vanessa* or *Pieris* exposed to the sun will walk in a straight line; if the ground on which they are walking is rotated through 90°, they will turn through the same angle; if they are covered with a dark box, they will turn in bends and spirals, but on exposure to the sun again they move in a straight line in the old direction (Fig. 149).[35] If *Geotrupes*, *Coccinella*, *Forficula*, *Dixippus*, &c., are walking in the presence of a single source of light from a given point, and this light is switched off as another is switched on, they will turn through the smallest angle possible so as to receive the new source of illumination from the same quarter.[18]

This oblique type of orientation to light has sometimes been regarded, for example in caterpillars,[63, 68] as an 'asymmetrical tropotaxis'; that is, the insect maintains equal activity on the two sides of the body only when there is a constant degree of inequality in the stimulation of the two eyes. In insects with primitive eyes capable of perceiving only differences in luminosity

this would be the only mechanism by which orientation could be maintained.[68] But we have seen (p. 126) that even caterpillars are capable of some degree of perception of form; and most authors consider that the response is, rather, an 'asymmetrical telotaxis', dependent on the insect keeping constant the incidence of the light rays on a given region of the retina. For example, if *Geotrupes* is moving obliquely in respect to one light and a second is switched on, it pays no attention to the second and remains orientated by the first.[47] The response probably depends therefore on the maintenance of a constant pattern of stimulation in the retina.

If a bright light is employed, the insect may be caused to turn as soon as the beam of light passes from one ommatidium to the next. Good agreement exists between the ommatidial angle and the smallest angle through which the light must be moved in order to cause a change in direction. This affords further evidence that the ommatidium is the visual unit. At low illumination, in *Geotrupes* and some other beetles, the light must be moved through a larger angle; it appears as though in dim light seven adjacent ommatidia become linked to form a new visual unit.[18]

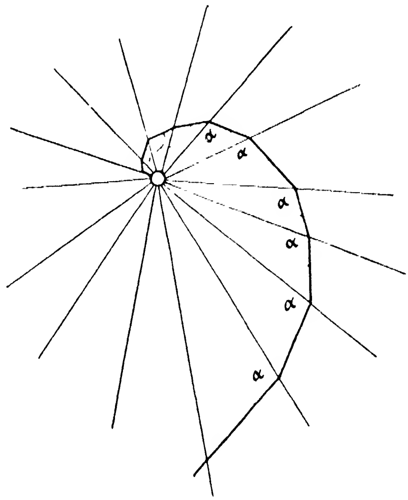

FIG. 150.—Menotaxis (*after* v. BUDDENBROCK)

An insect moving so that its course makes a constant angle and with the beam of light from a near source approaches the light along a logarithmic spiral.

In this type of orientation, if the source of light is distant, like the sun or moon, the insect will continue in a straight line. But if the light is close, its incidence on the retina will soon change if the insect goes straight. The angle of incidence of the light can be kept constant only if the insect continually turns towards the source. It will thus move along a logarithmic spiral ending at the light itself (Fig. 150).[15] The flight of moths to a flame is explained on these lines; the insect utilizes a near source of light for a mechanism of orientation which is adapted only to distant sources.[17] Caterpillars will sometimes crawl to a light in exactly this type of spiral.[68]

Menotaxis—Insects may orientate themselves obliquely not merely to a bright point of light, as in the light-compass response, but to more or less complex patterns. The basis of all such responses appears to be the same: the maintenance of a constant visual pattern. They are commonly grouped together under the term 'menotaxis'.

As the result of such photic orientation, insects placed on a turn-table move round when this rotates; they will often remain, for example, facing a window or other conspicuous object.[81] They will show the same response, also, if they themselves are stationary and a striped screen is moved past them ('optomotor response').[87] If stripes are moved underneath a resting fly (*Pollenia*) it will take up an attitude, recalling that of *Proctacanthus* with partially covered eyes, reaching out in the direction of movement (Fig. 151).[32] Aquatic insects such as *Aeschna*,[84] *Dytiscus*[118] or *Notonecta*[89] will swim around

a glass container if vertical stripes are rotated around it, keeping themselves in line with a given stripe.

This 'optomotor response' appears to be a reaction to changes in the general visual pattern, not to a single point, and it is effected through the lateral and peripheral regions of the eye. It is quite distinct from, and is, indeed, inhibited by the 'fixation response', which is a reaction to single points falling on the region of most acute vision.[101]

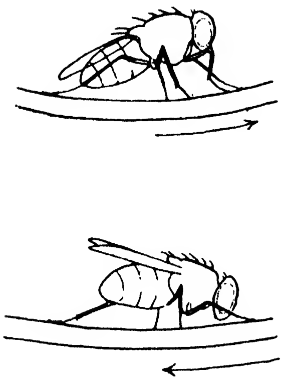

FIG. 151.—Attitudes taken up by the fly *Pollenia* when a striped pattern is moved below it in the direction of the arrows (*after* GAFFRON)

By the same mechanism *Notonecta* can maintain itself at a fixed point in a flowing stream: it turns and faces the current and swims so as to keep constant the visual impressions it receives. If landmarks on the bank are moved it will move with them; if the eyes are blackened, in the dark, or in a vessel with plain white sides, the reaction fails and the insect is carried down-stream In this visual response, movement across the eye from behind forwards offers the greater stimulus; were this not so, active movements of the insect forwards would be inhibited.[89] *Dytiscus* with one eye covered makes circus movements towards the uncovered side; these movements can be arrested or even reversed by the orientation to revolving stripes.[118] Even Gyrinid beetles swimming in all directions in the water surface have been shown to maintain their position in flowing water by means of the surrounding pattern; although in this case the pattern of retinal stimulation is continually changing. Swarms of Gyrinids are completely disorientated in the dark or if a sudden change in landmarks is made.[12] The orientation against a current of water ('rheotaxis') is thus in fact a visual response. The same applies probably to insects flying in a current of air ('anemotaxis'), though this has been little studied as yet.*

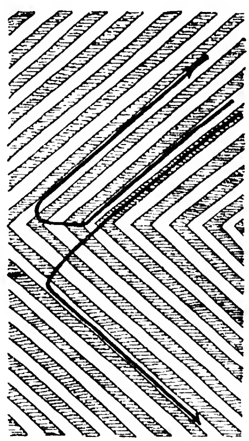

FIG. 152.—Tracks followed by *Coccinella* on a pattern made up of black and white stripes (*after* TISCHLER)

The tendency for insects to walk along black and white stripes, or along the border of a wide black stripe, even when set obliquely in a vertical surface, which is seen in *Lymantria* larvae,[48] *Dixippus*,[52] *Coccinella*, &c.,[99] is perhaps in part menotaxis of the same kind (Fig. 152). The circus movement due to covering one eye in *Coccinella* can be eliminated in the presence of such a pattern.[99]

Skototaxis—Various insects exposed in a diffusely lit arena with a black screen in it, will orientate themselves towards the black screen; and among a series of grey screens they will make for the darkest. This response is termed 'skototaxis'. It has been observed in *Lepisma*,[73] *Forficula*,[58] caterpillars,[35] and the ant *Lasius*.[114] If *Forficula* is offered a dark screen and a

* *Stomoxys* and *Lyperosia* [61] will sit at rest facing a current of air. This is presumably a tactile response.

single light, two modes of orientation may be made to compete, with the result that the insect may either go directly away from the light (negative phototaxis) or towards the screen (skototaxis) (Fig. 153).[58] This response demands a discrimination of luminosity in different parts of the field; that is, a perception of form or pattern. In some cases it can be shown that form is important in the orientation; for example, the small Carabid *Notiophilus* will ignore a black screen measuring 15 × 15 cm. near to it, and make for a screen measuring 4 × 4 cm. some distance away.[114] We have already seen that caterpillars prefer dark vertical forms of particular proportions (p. 126).

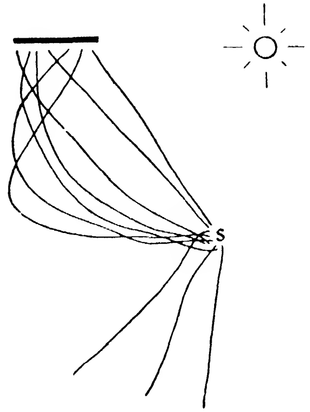

FIG. 153.—Tracks followed by *Forficula* in presence of a single source of light and a small black screen. Some insects show skototaxis; others show negative phototaxis (*after* KLEIN)

Dorsal light reflex—Certain aquatic insects, such as Ephemerid nymphs and Dytiscid larvae, maintain their position in the water by orientation to illumination from above. This is termed the 'dorsal light reflex'.[17] A nymph of *Cloëon* illuminated from below is caused to turn over on its back. This response persists if one eye is blackened; suggesting that it is a menotactic response. It is inhibited if the feet are in contact with some object (thigmotaxis).[2]

Orientation by temperature—The avoidance of high temperatures which is shown by all insects is clearly an example of 'phobotaxis': as soon as the insect comes in contact with an unfavourable temperature it makes a reflex 'avoiding reaction' and turns away. The orientation of blood-sucking insects by the warmth of their host is a different problem. The importance of temperature for this purpose has been recognized in many such insects (p. 152) but the mechanism of orientation has been studied only in the blood-sucking bugs *Rhodnius* [115] and *Cimex*.[91] It has been proved in *Rhodnius* that it is the air temperature and not the radiant heat which is the source of stimulus: this bug is attracted equally by a test-tube full of warm water and by a similar tube covered with aluminium foil—although, as may be shown with a suitable thermopile connected to a galvanometer, the radiant heat emitted by the metal-covered tube is less than one-tenth that from the other. Placed at a distance of 4 or 5 cm. from a test-tube at 37° C., *Rhodnius* first cleans the antennae, then moves them in each direction in turn, and finally extends them towards the warm tube, advances to it and probes it with the labium. It makes exactly the same response and locates the tube in the same way if one antenna is removed. The stationary insect is thus able to judge with its antennae or with a single antenna in which direction the source of stimulus lies (Fig. 154). For that reason the response has been compared to the telotactic visual response.[115] But the insect is not in fact reacting to the distant stimulus but to the warm air within the immediate range of its antennae. The exploratory movements of the antennae which precede the movement of the insect and determine its direction have therefore been compared to the 'trial and error' response or 'phobotaxis'.[91] The louse *Pediculus*, offered two fingers,

often advances along the resultant between them, suggesting a 'thermotropotaxis'.[46]

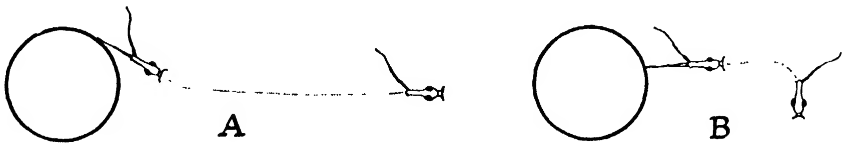

FIG. 154.—Course followed by *Rhodnius* with a single antenna in the presence of a test tube of warm water

In *A*, the insect inclined abruptly towards the intact antenna when close to the source of stimulus (*after* WIGGLESWORTH and GILLETT).

Orientation by smell has much in common with orientation by temperature; indeed in blood-sucking insects the two factors are concerned at the same time. In both cases the insect perceives with its antennae the qualities of the air in its immediate vicinity, and by means of these perceptions it is able to reach the source of stimulus. The insect is at first excited by the attractive odour whether from food or from the opposite sex; the scent has a stimulatory or kinetic effect. In this state it may move hither and thither and eventually reach the source by 'phobotaxis' or trial and error.[86] *Drosophila* deprived of the wings, in the presence of a strong source of odour such as fermenting fruit, with a concentration gradient in still air, can steer in a straight line towards it from a distance of 15–20 cm.[4, 24]; *Habrobracon* which locates its host, *Ephestia* larvae, by smell, advances to it in a straight line over the last 5 cm.,[76] and the same applies to the beetle *Geotrupes* in the presence of dung.[110] Apparently the excited insect will continue in a straight line if the gradient of stimulation is increasing, but turns aside if the gradient falls. The mechanism in these cases is described as 'phobotaxis'.

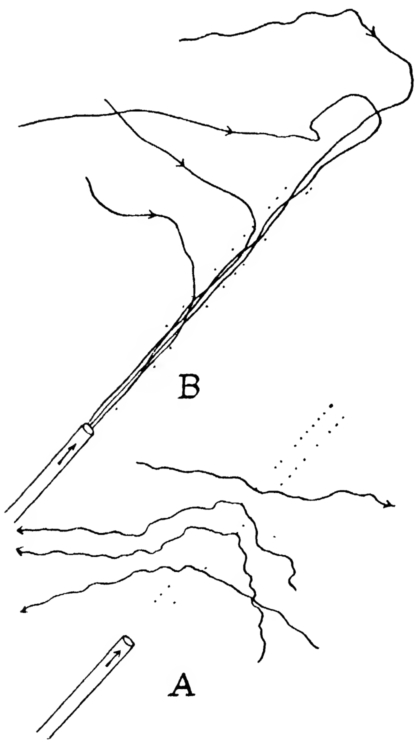

FIG. 155.—Tracks followed by *Drosophila* deprived of their wings when exposed to a current of air

A, odourless stream of air; *B*, air carrying odour of pears. (*After* FLÜGGE.)

But in the course of this movement, orientation may perhaps be assisted by the relative intensity of stimulation in the two antennae. For if one antenna be removed in *Drosophila*[4] or *Calliphora*[38] in the presence of food, or in males of *Bombyx mori*[54] or *Tenebrio*[107] in the presence of the female, the insect turns more frequently towards the intact side; and *Geotrupes* so treated inclines towards that side when it gets close to the dung,[110] as does *Rhodnius* as it approaches a source of warmth and smell.[115] While in the presence of a

repellant odour *Drosophila* with one antenna turns chiefly to the operated side.[24] The orientation is therefore sometimes said to be in part tropotactic. But whether such differential stimulation of the antennae is important in the normal insect is uncertain; for the difference on the two sides can never approach that caused by complete removal of one antenna; the gradient of smell must usually be greatly upset by air currents; and in insects with mobile antennae capable of testing the air on all sides, such as *Habrobracon*[76] and *Rhodnius*,[115] the insect can orientate itself to the source of smell almost equally well with one antenna. From a distance of 5 cm. *Rhodnius* can determine the direction of a source of warmth and smell (a warm tube of water covered with fresh mouse skin) before moving, by reaching out with its antennae; it will then advance straight to the source, sometimes inclining towards the intact antenna when close to it. The mechanism of this response has already been discussed (p. 169).

It is possible in some cases that the current of air bearing an attractive odour may assist in orientation. *Geotrupes* generally approaches dung up-wind, detecting it from a distance of 2–4 metres.[110] *Drosophila* deprived of the wings turns sharply into such a current of air and moves rapidly and accurately against it. It is merely disturbed without being orientated by an odourless stream (Fig. 155).[24]

Another method of orientation by odours consists in following trails of scent. This is extensively used by ants which, as they go along, repeatedly touch the surface with the abdomen and so leave a delicate trail of formic acid. If artificial routes are made by painting the earth or plant stems with dilute formic acid, ants can easily be led astray along them.[17] Similarly, the ovipositing female of *Habrobracon* may find the larva of *Ephestia* by following its trail, bringing the antennae closely in contact with the ground.[76]

CO-ORDINATED BEHAVIOUR

In the experimental study of orientation, conditions are usually so arranged that the insect receives only one kind of stimulus at one time; the response then appears as a taxis or 'forced movement'. But under normal conditions the insect is exposed to a great variety of stimuli which compete with one another in the production of reactions. According to some authors (the 'Gestalt' school) the organism responds to changes in the whole complex or pattern of stimuli to which it is exposed, and hence it is impossible to obtain a true description of behaviour by considering responses to single stimuli. But the physiological study of behaviour is necessarily analytic, and in this section we shall endeavour to indicate some of the ways in which normal behaviour can be resolved into a succession of responses of the sort already considered in the present chapter.

Relation of simple responses to biology—The newly hatched larvae of *Euproctis* or *Lymantria* show two dominating reactions: a negative geotaxis and a positive phototaxis. These responses ordinarily serve to lead them up to the leaves of their food plant. Of the two reactions the phototaxis is the stronger and if, under experimental conditions, they are illuminated from below, they will migrate downwards and die of starvation.[63, 65] The newly hatched larva of the weevil *Sitona lineata* shows no directed responses to

light, but it is positively geotactic and very strongly thigmotactic; these responses cause it to burrow in the soil.[3] Larvae of the Elaterid *Agriotes*, which also burrow in the soil, show a photokinesis and a negative phototaxis in addition to geotaxis and thigmotaxis.[94] *Forficula* shows photokinesis, thigmotaxis, hygrokinesis, and negative geotaxis; responses which will explain the normal hiding places of earwigs, and are utilized by gardeners when they trap earwigs in flower-pots containing dry straw inverted on canes.[112] Many other examples of the same kind could be quoted.

Succession and inhibition of responses—How varied are the perceptions which bring about even a simple reaction is shown by the feeding behaviour of blood-sucking insects.[61] In *Stomoxys* and *Lyperosia*, for example, movement to the host is determined by smell from the skin, warmth, and moisture; extension of the proboscis by the same stimuli at close quarters; probing, by smell and warmth; sucking, by taste.[61] Reactions of this kind consist of series of responses each of which prepares the nervous system to react to the next. A simple example of this is seen during the feeding response of *Rhodnius*. Normally, if the antenna of *Rhodnius* is touched by some cold object it is quickly withdrawn and the insect retreats. But if it is touched by such an object during the feeding reaction, while the insect is advancing towards a source of stimulus, the proboscis is at once extended—this being the ensuing response in the reflex chain.[115]

In the feeding of butterflies, the flower scent sometimes merely excites the insect, which then uses the coloured object as a visual mark: smell from a distance, vision, and smell or taste at close quarters are all concerned in different proportions. In some butterflies the colour stimulus alone may initiate the whole reflex chain of the feeding reaction.[49] A greenish-yellow colour will attract the ovipositing female of *Macroglossa*, but only if the odour of *Galium* is added to the visual stimulation is egg-laying started: eggs are immediately deposited on a test-tube filled with a solution of chlorophyll and covered with *Galium* extract.[59]

The multiplicity of perceptions may have another important effect; responses to stimuli received through one set of sense organs may be inhibited by the perceptions of another group. An example of this may again be taken from the behaviour of *Rhodnius*. In the normal bug it is very difficult to elicit the probing reaction by visual stimuli, because objects moved in front of the eye set up air currents perceived by the antennae and the insect moves away. But if the antennae are removed, it is possible to obtain a very striking response; the bug will run in all directions after a moving pencil and probe it immediately the movement ceases.[115]

Sometimes, during response to the perceptions of one sense the perceptions of another sense seem to be centrally inhibited. An example of this is afforded by the hunting wasp *Philanthus*, which stocks its nest solely with honey-bees. It hunts these bees by sight, and is often attracted momentarily to bumble-bees or other insects. It may perceive a bumble-bee at 30 cm. distance, but on approaching within 5–15 cm. it is recognized by its smell and left; whereas a honey-bee is seized. Smell is therefore exceedingly important in obtaining prey; but it is brought into play only when the presumed victim has been seen; during the process of searching *Philanthus* is completely insensitive to bee odour.[98]

Succession of responses is a characteristic also of the building behaviour of solitary bees and wasps. Each act in this process can be performed only in its proper sequence. The succession of duties discharged by workers of the honey-bee, at first in the hive and later foraging, is perhaps another example of the same phenomenon in its most complicated form; though these duties can be altered to some extent, abbreviated or extended beyond their time, according to the needs of the community.[31, 83]

Route finding by Hymenoptera—The methods used by Hymenoptera in finding their way to and from the nest furnish one of the best examples of the co-ordination of multiple impressions. Ants which forage far from the nest utilize many factors: the direction of the sun's rays (' light-compass ' orientation), the visual perception of landmarks, the odour of trails, a topochemical sense (by which is meant the perception of scents to either side of a given track,

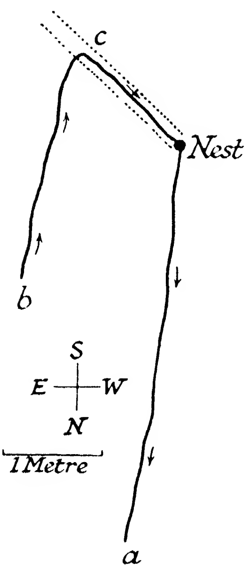

FIG. 156.—Finding of nest by worker of *Messor barbarus* (*after* SANTSCHI)

For explanation see text.

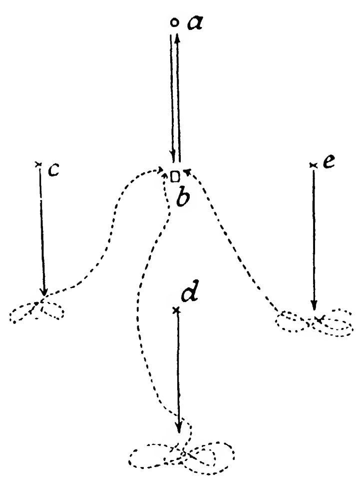

FIG. 157.—Return of bees to the hive (*after* WOLF)

Bees feeding at *a* fly straight to and from the hive at *b*. If removed from *a* to *c*, *d*, or *e*, they always fly in the same direction and for the same distance before returning to the hive.

perceptions which will be reversed on the return journey), tactile sense, proprioceptive senses and perhaps fatigue.[85] Fig. 156 illustrates a simple experiment with the ant *Messor barbarus*. It went north from the nest to *a*; it was then displaced to *b*, and orientating itself by the direction of the light it returned south to *c*. Here it encountered a known track which it followed to the nest, the direction of the nest being determined probably by distant landmarks and by topochemical perceptions.[85]

Bees likewise make use of many factors in returning to their hive. In a new locality or on an open sheet of water without landmarks the direction of the sun's rays is the chief factor. But they can also appreciate the *distance* from the collecting ground to the hive (Fig. 157). Later they make use of landmarks and are able to remember and utilize the angles through which they have to turn at particular points. In open country they can be made to follow

a series of artificial landmarks. In the immediate vicinity of the hive, orientation marks are used ; if the hive is moved, conspicuous landmarks will accelerate the accumulation of a crowd of bees at its original site. But scent is also important, for if the bees are accustomed, for example, to a scent of aniseed in the hive, they will locate it in its new position considerably sooner.[116]

Memory and conditioned responses—The greater part of behaviour in insects is inborn, being made up of responses which are performed with the same precision on the first occasion in the life of the individual as later. But to some extent behaviour can be influenced by experience. The simplest example of this is termed 'motor learning'. If *Ephestia* larvae are kept walking round a circular dish for ten minutes or so, they will continue to move in an arc when set free, even in a beam of light in which they normally go straight; and if they are placed in a track with a series of T-shaped turnings and forced always to turn the same way at the bends (Fig. 158), they will continue such turns when given a free choice.[9]

But learning can also result from an association of sensory experiences. The cockroach normally avoids the light and seeks the dark ; but by setting up a chamber with light and dark ends, and giving the insects electric shocks at the dark end, they can be taught to remain in the light section, and return there from the dark section, without repeating the shocks. Different individuals require from 18 to 118 shocks before they acquire this change in response. They retain the change for 4–55 minutes, but acquire it more rapidly a second time.[95, 102] If such an experiment is arranged in the form of a T-shaped maze in which electric shocks are given on one side and a dark shelter provided on the other, *Periplaneta* can be trained to turn always to the right or the left. This result is not affected by blinding ; but removal of the antennae causes immediate loss of the training ; and after removal of the left antenna an insect trained to go to the left almost always turns to the right. The cockroach clearly depends on the antennae for the appreciation of right and left.[11]

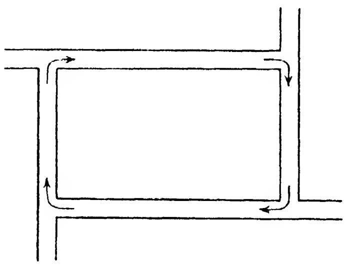

FIG. 158.—Simple maze consisting of successive T turnings in which larva of *Ephestia* is obliged always to turn to the right (*after* BRANDT)

This type of association, where the insect is 'punished' after responding to a given stimulus, is sometimes termed 'negative' training, as opposed to 'positive' training in which it is 'rewarded' after a particular response. Both methods have been used in *Dytiscus*, which may learn to associate a given taste or scent with bitter meat to follow (negative training) or with sweetened meat to follow (positive training). Positive training is generally found to give results more rapidly.[37, 86]

The length of time required to produce such responses and the time they are retained varies with the species and with the senses employed. *Dytiscus* can retain the effects of teaching for some days.[86] Butterflies require several days' training before they will associate particular colours with the presence of food, and after an interval of one day they retain only traces of the acquired response[49]; whereas bees may be successfully trained to come to a given colour within 2 hours and retain this training for 4 days.[29] And they are

still more retentive of odours: a scent used for training for only a few hours may be remembered after several weeks.[30] It is obvious that memory, both visual and olfactory, plays a large part in the finding of the way to and from the nest among Hymenoptera (p. 173).

It has been proved experimentally that in associating a given colour with a source of food, the bee uses only those perceptions which *precede* its discovery—those stimuli which it receives during the 'approach flight' occupying perhaps 3 seconds. It is unaffected, for example, by any changes made in the colour of the background while it is feeding, or by the colour present during the longer 'orientation flight', occupying perhaps 10 seconds, which precedes the return to the hive. This is precisely what happens in the so-called conditioned reflexes of mammals. But if the food is removed to a new site during feeding, the bee usually returns to the place where feeding was finished, that is, the site 'memorized' during the orientation flight.[77]

A special kind of 'memory' is seen in the oviposition responses of some insects. The Ichneumonid *Nemeritis canescens* during oviposition shows a strong preference for the odour of *Ephestia* which it normally parasitizes; but this preference can be reduced if it has been reared in larvae of the wax moth *Meliphora grisella*.[97] And similar responses are seen in some leaf-eating caterpillars and saw-flies in which the oviposition site of the female is influenced by the nature of the food during larval life.[106]

Appreciation of time—Bees can appreciate time and can be trained to come for food at any hour of the day (Fig. 159).[5] If the concentration of sugar

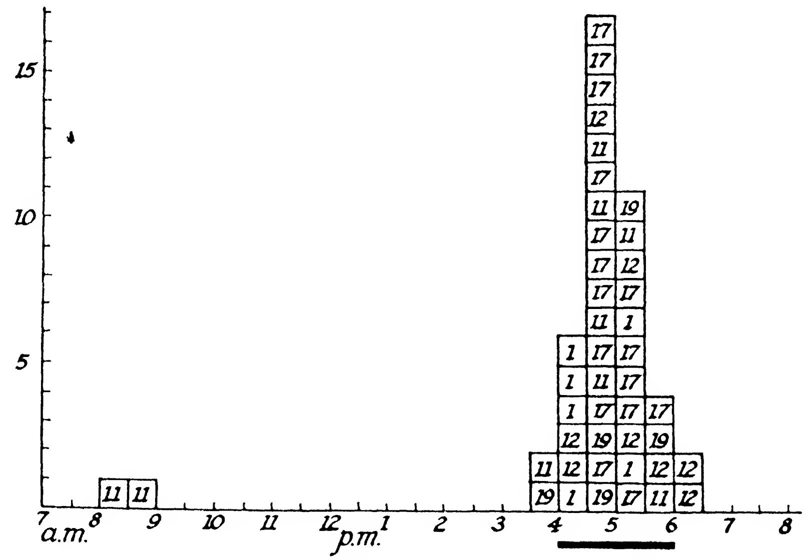

FIG. 159.—Appreciation of time by the honey-bee (*after* BELING)

Bees were given syrup at an artificial feeding place for 3 weeks between 4 and 6 p.m. On the day of experiment the syrup was left out all day. Ordinate: number of bees visiting the feeding place during half-hour periods. Abscissa: time of day. The numbers in the squares indicate individual marked bees.

which is available differs at different times of day, they are able to remember the time at which it is greatest.[109] Under natural conditions this memory for time is connected with the fact that the flowers visited offer their pollen

or nectar at particular hours.[57] Wasps, also, have some appreciation of time [108] and it is claimed that ants can remember periods ranging from 3 hours up to 5 days.[36]

The nature of such memory for time is uncertain. In the bee it seems to be bound up with the 24-hour rhythm; if bees are fed at a 48-hour period, they come for food also at the 24-hour intervals.[5, 31] Yet it appears to be independent of periodic changes in the outer world; bees can still recognize the hour of day if the illumination is kept constant artificially.[5] It is possible that they are perceiving some periodic factor in the environment as yet unrecognized; but the evidence seems to point to their depending on metabolic changes in their own bodies. For if bees trained to come for food at a given hour are kept cold, their time of arrival is delayed; whereas narcosis is without effect.[36, 51] Bees firmly trained to forage at a particular hour spend the rest of the day in a remote part of the hive. Here they are not disturbed by the dancing of bees which have discovered a rich source of nectar or pollen. These discoverer bees excite, attract and lead out only those foraging bees whose habits have not become fixed in this way.[31]

Diurnal rhythms—Many of the activities of insects show a diurnal periodicity. This is seen in the movements of the iris pigment in Noctuids (p. 113), and in the general colour change in *Dixippus*, which is light by day and dark by night (p. 337). *Dixippus*, also, is active at night and in a state of catalepsy by day; a rhythm which may persist for some days in permanent darkness or even with reversed illumination,[93] and it shows a daily rhythm in defaecation and oviposition.[53] The fire-fly *Luciola sinensis* kept for several days in the dark will still light up at the normal time of 7 p.m.[92]; and *Photinus pyralis* shows periods of flashing which recur at intervals of 24 hours and persist for at least 4 days in the uniform conditions of the darkroom.[14] *Gryllus* shows a daily rhythm in general activity which will persist at least two weeks in continuous darkness at constant temperature and humidity. Reversed illumination will cause reversal of activity which, again, will continue under constant conditions.[69, 78] All such rhythms appear to indicate a memory for time; but they are usually regarded as being dependent on the rate of metabolism in the cells,[19] which results perhaps in a rhythm of hormone secretion.[53]

A somewhat different type of rhythm is seen in the emergence of insects from the pupa, or in moulting, or hatching from the egg, as in *Dixippus*.[53] Most species emerge at a definite time of day, and will continue to do so even when those factors (temperature, light, barometric pressure, relative humidity, electrical potential and electrical conductivity of the air) which show a daily periodicity, are kept constant. *Ephestia* kept in a constant temperature room for three generations continue to emerge chiefly in the evening. But this inborn rhythm can be overcome by external stimuli. When there is a diurnal cycle of temperature, emergence in *Ephestia* occurs when the temperature begins to fall (Fig. 160); and if larvae or pupae are exposed to an artificial rhythm of this kind, emergence can be induced at an abnormal time of day. Such a rhythm may be produced if the period of the cycle is 16 hours or 20 hours, but not if it exceeds 24 hours; evidently there is some close relation with the normal daily period.[90] In *Drosophila*, which emerges chiefly in the morning, the periodicity disappears after rearing for 15 generations in uniform

dimly lit conditions ; but a single exposure to daylight is sufficient to initiate a diurnal periodicity in emergence. Here again the metabolic rate seems to

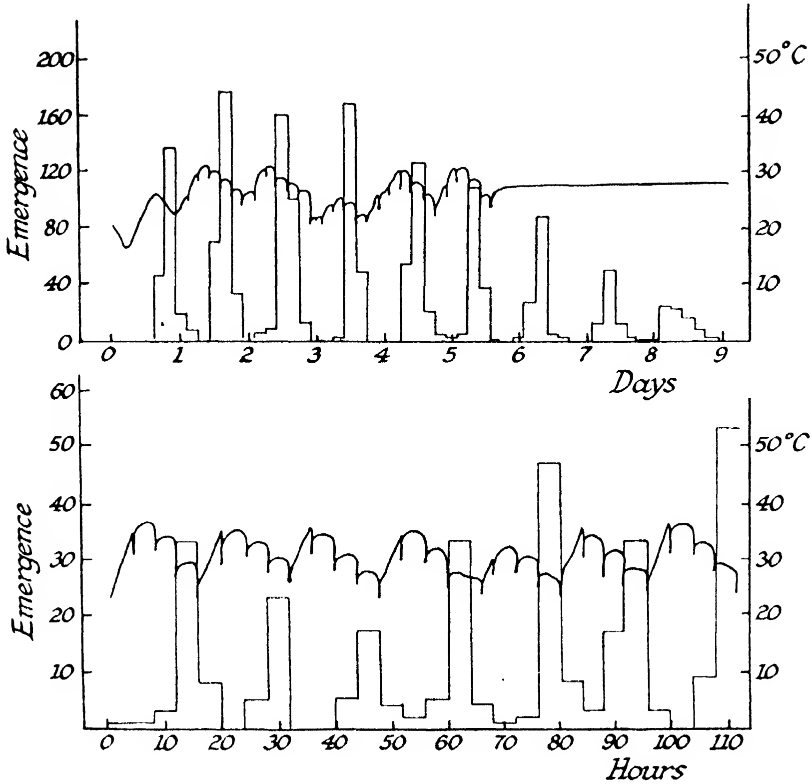

FIG. 160.—Rhythm of emergence in pupae of *Ephestia kuhniella* (*after* SCOTT)

Ordinates : to the left, number of insects emerging during a given period (indicated by the blocks in the figures) ; to the right, temperature in ° C. (indicated by the continuous line). Abscissae : upper chart, d[illegible] [illegible]ower chart, hours.

Upp[illegible] [illegible]rt shows 24-hour temperature rhythm. Maximum emergence occurs when the temperature be[illegible]ns to fall and this emergence rhythm persists with constant temperature. Lower chart shows 16-hour rhythm of temperature and emergence.

lie at the base of this apparent memory. The length of period can be prolonged to 30 hours by exposure to 10° C. and prolonged to 30–35 hours if the oxygen tension is reduced to 5 per cent. of the normal.[19]

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Chapter IX

Respiration

IN THIS chapter we shall consider how oxygen is conveyed from the atmosphere to the tissues, and how the carbon dioxide resulting from oxidation in the tissues is eliminated. The original respiratory organ of all metazoa is the skin; but we have seen that the skin of insects, in association with their terrestrial existence, has become impermeable to water and thereby ill-adapted for respiration. The great majority of insects, as was shown by Malpighi (1669) in the silkworm, breathe by means of tracheal tubes, which usually open at the surface of the body through a number of spiracles, and convey air directly to the tissues. Respiration solely by the skin, without the intervention of an air-filled system of this type, has been acquired, probably secondarily,[127] by a few of the primitive Poduridae from moist surroundings, and in the young stages of some aquatic and parasitic forms (p. 203).

THE TRACHEAL SYSTEM

The tracheae are invaginations of the cuticle, which branch everywhere among the tissues. In the primitive Campodea each spiracle gives rise to a tracheal tree which is quite independent, and shows no anastomoses with the tracheae of adjacent segments; but in all other insects the tracheae anastomose freely to form longitudinal and transverse trunks (Fig. 161). With the exception of some Collembola living in wet places, and in the larvae of some insects (the bee, many Diptera), the spiracles are almost always provided with closing mechanisms of varied design—first observed by Burmeister (1832).[6, 81, 88, 157]

Structure of tracheae—The histological structure of the tracheae is essentially the same as that of the body surface from which they are derived; they consist of a matrix of discrete epithelial cells, relatively thick near the spiracles, flattened along the deeper branches, and a thin cuticular lining (Fig. 162). The cuticle is thrown into folds, which typically run a spiral course round the tubes for a short distance before a new fold begins. Often the margins of the folds fuse to form a thread, the so-called spiral filament.[43] Near the spiracles the folds are often irregular and sometimes hardly more than a collection of wrinkles in the crumpled membrane.[136] Branched projections may run laterally from the folds so that the cuticle shows quadrangular areas.[43, 97] Sometimes the spiral thread gives off hair-like processes into the lumen of the trachea.[43, 136]

As already noted (p. 18) the cuticle lining the tracheae has the same composition as that covering the surface of the insect and the ducts of the dermal glands. Near the spiracle the lining of cuticulin has a thick layer containing chitin beneath it. In the smaller branches and the air sacs chitin is absent. In the finest terminations the lining membrane is excessively delicate, and is freely

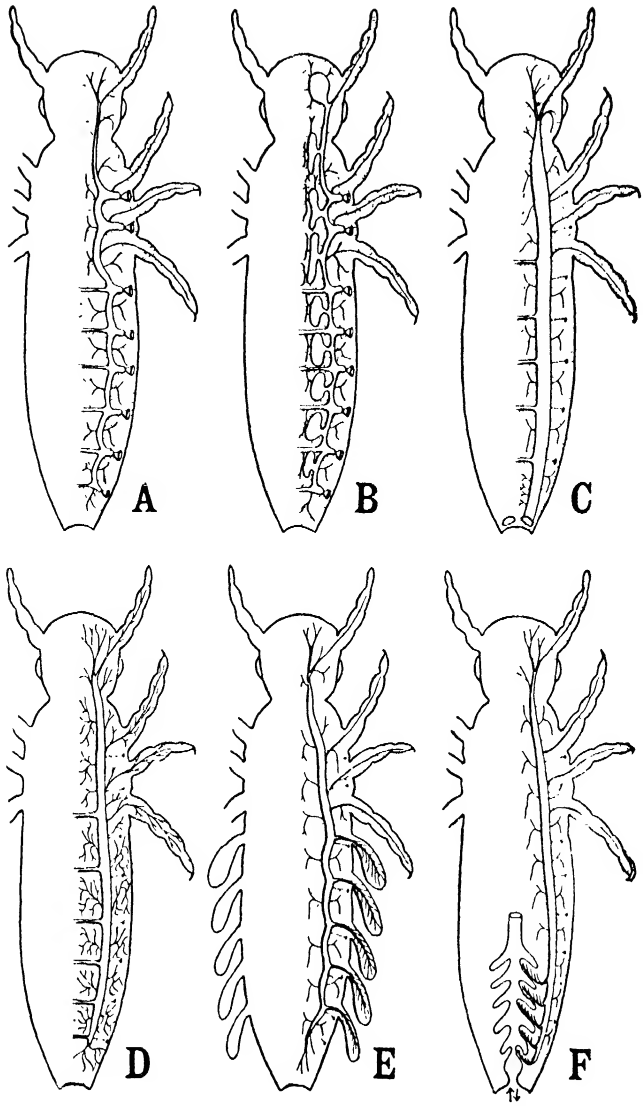

FIG. 161.—Some types of respiratory system in insects, schematic

A, simple anastomosing tracheae, with sphincters in the spiracles ; *B*, mechanically ventilated air sacs developed ; *C*, metapneustic respiration, terminal spiracles alone functional ; *D*, tracheal system entirely closed, cutaneous respiration ; *E*, the same with abdominal tracheal gills ; *F*, the same with rectal tracheal gills.

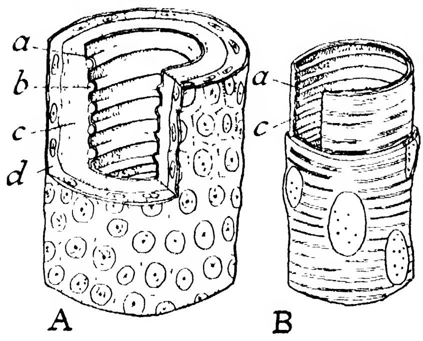

FIG. 162.—Structure of tracheae (*after* WEBER)

A, tracheal branch close to spiracle ; *B*, small tracheal branch more highly magnified. **a**, epicuticle thrown into spiral folds ; *b*, exocuticle ; *c*, endocuticle ; *d*, epithelial matrix.

permeable to water.[165] Its composition is not known, but it is worth noting that during moulting and metamorphosis this membrane is dissolved completely.[145]

Types of tracheae—In form the tracheae are extremely varied.[21] Typically they are circular in cross section and prevented from collapsing by their spiral folds. Often they are elliptical, as in the main tracheae of *Dytiscus* larvae,[118] or mosquito larvae,[7] and the 'spiral thread' then tends to atrophy so that the tubes collapse when the air pressure within them is reduced. Saccular dilatations may occur along their course, as in *Melolontha*; and such dilatations may be enlarged by the fusion of the matrix of adjacent branches to form great air sacs,[42] as in *Orthoptera*, *Dytiscus*, *Apis* (Fig. 163), *Musca*, &c., and become bound to the surrounding tissues, usually muscles, by numberless small branches which they give off. These air spaces are generally flattened and often collapsed; but if they are so placed that they cannot collapse, as in the head or parts of the thorax, they form permanent air chambers. Nerves or muscles may run across such chambers and they are then invested with the tracheal coverings, but in the reverse order, with the cuticle outside ('inverted tracheae').[70]

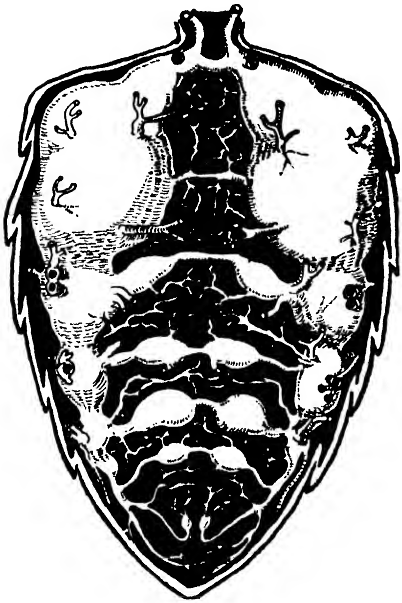

FIG. 163.—Tracheal system in the abdomen of the honey-bee worker, showing the air sacs. Dorsal tracheae and air sacs have been removed (*after* SNODGRASS)

Tracheal endings—The mode of ending of the tracheae shows an equal diversity.[79] Typically, when the trachea has been reduced by repeated branching to a diameter of 2–5μ it enters a large stellate cell, the 'tracheal end cell' or 'transition cell', [66] and there breaks up abruptly into a number of tracheal capillaries or tracheoles, which are less than 1μ in thickness and are characterized by the absence of a spiral fold (Fig. 164). These cells are probably homologous with the epithelial cells that lay down the tracheal cuticle; they have become spread out into a web-like form through being situated at the point of furcation, and they are very large because they have to look after so many branches.[96, 164] When the trachea passes gradually into the tracheole, as is the case at many tracheal endings, tracheal end cells are not conspicuous [16, 35, 55]; nor are they where a single tracheole arises from the side of a large tracheal stem.

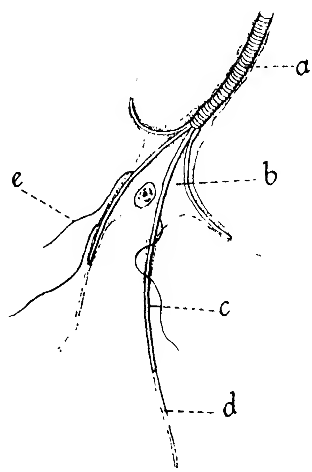

FIG. 164.—Tracheal end cell in head of mosquito larva *Aëdes aegypti* (*after* WIGGLESWORTH)

a, trachea; ***b***, tracheal end cell; ***c***, tracheole containing air; ***d***, terminal part of tracheole containing fluid; **e**, fine branch of tracheole.

These palmate tracheal cells may unite with one another by means of the finger-like processes

they give off, and so form a fenestrated membrane, rich in tracheae, in which the organs are invested. That is so around the ovaries and testes.[124, 126, 133] It is this membrane, variously modified, which forms the 'peritoneal membranes' and indefinite connective tissues of insects, as around the intestine.[42] It is said that the membranes so formed may be differentiated to form muscle fibres and elastic fibres.[124, 126]

In many tissues, membranes of this kind cannot be detected. In the salivary glands and gut of some insects the tracheoles ramify to form a rich network around and between the epithelial cells without penetrating the cytoplasm.[24, 154, 174] In other insects or other tissues the tracheoles enter the cells—as in the photogenic cells in the luminous organs of beetles,[96, 164] the fat body sometimes,[144] in the cells of the *organe rouge* of *Gastrophilus* larvae,[120] the salivary glands of Hemiptera,[48] in the rectal glands of Muscids, and the anal papillae of mosquito larvae.[167] Much of the controversy that has centred round this question is doubtless due to the fact that there is great variation in different organs.[166]

There are many differences, also, in the mode of ending in the muscles. In the ordinary muscles of the limb and body wall the tracheoles lie on the surface, or penetrate a short distance into the superficial layers of the fibre—as in the leg muscles of *Hydrophilus* [5, 72a] or the muscles of the bee, excluding the flight muscles.[67] On the other hand, in the flight muscles, there is often a network of intracellular tracheoles running and anastomosing chiefly longitudinally [5, 72a, 105]; and in wing muscles of the fibrillar type (p. 78) the tracheoles ramify in the substance of the sarcosomes, closely investing each fibril [5] so that each sarcomere is ringed almost completely by a tracheole or by portions of adjoining tracheoles.[108]

It is uncertain whether the tracheoles commonly end by anastomosing with one another.[166] There seems little doubt that they may do so within the muscle fibres [105]; but they certainly end separately as a rule in the fenestrated membranes on the surface of organs [124, 126]; and even in the gill plates of *Aeschna* nymphs, where they appear to form closed loops, careful observation has shown that each capillary ends blindly.[78]

Movements of fluid in the tracheal endings—After death the tracheoles quickly fill with fluid, which creeps along them from the tissues. This has led to an old-standing controversy as to whether, during life, the endings contain air or liquid.[166] The question cannot be decided by dissection of the tissues, because they are so easily affected by the fluid in which they are immersed. But examination of living insects under the microscope by transmitted light has shown that there is much variation in different tissues and different insects. In the abdomen of the flea, for example, the column of air in the tracheoles ends abruptly while they are still quite large (perhaps 0·2–0·3μ) and beyond that they contain fluid; whereas among the muscles of the legs they contain air as far as they can be resolved with the microscope. In the young mealworm larva (*Tenebrio*) the air extends further among the muscles of the body wall than on the surface of the gut (Fig. 165, A) [165]; while in the Anoplura all the tracheoles seem to contain air as far as they can be traced.

The tracheoles are so fine that the force of capillarity tending to draw liquid up them must be very great. Making certain assumptions it has been calculated that in a tube of 0·3μ diameter it is of the order of 10 atmospheres

pressure. The liquid must therefore be held at the tracheole ending by a force of at least this magnitude.[165] It is probable that imbibition by the colloid substance of the tracheole wall and the cytoplasmic layer around it is responsible for holding the liquid back, and that it is the differences in these properties in different organs and insects which are responsible for the differences in the extent to which water rises up the tracheoles.[169]

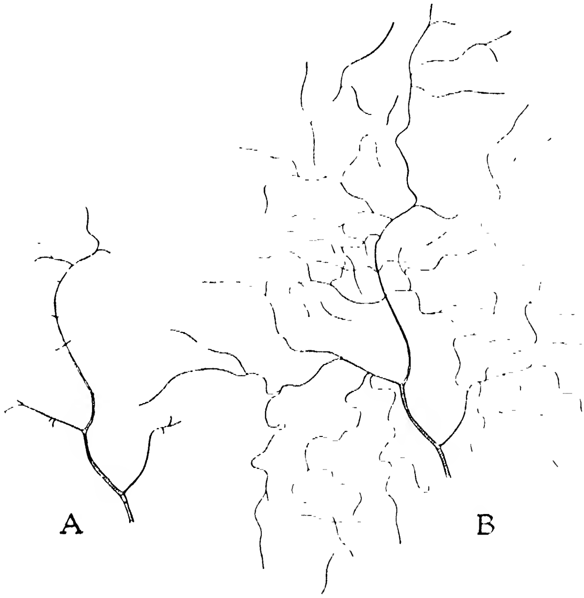

FIG. 165.—Tracheal ending on surface of gut of mealworm larva

A, at rest; *B*, after asphyxiation by flooding with water (*after* WIGGLESWORTH). The tracheoles are visible as black threads when they contain air, invisible when they contain fluid.

The water-binding power around the endings is influenced by changes in the osmotic pressure of the blood in which they are bathed. During muscular contraction, especially if the oxygen supply is deficient, the osmotic pressure

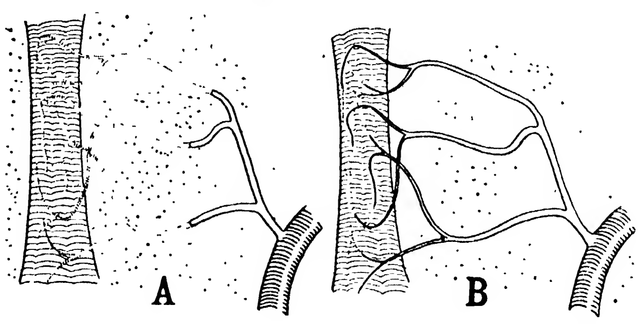

FIG. 166.—Tracheoles running to a muscle fibre: semi-schematic (*after* WIGGLESWORTH)

A, muscle at rest; terminal parts of tracheoles (shown dotted) contain fluid. *B*, muscle fatigued; air extends far into tracheoles.

of the blood increases (the osmotic pressure in the mosquito larva, expressed by the equivalent concentration of sodium chloride, will increase from 0·85 per cent. to 1·0 or 1·1 per cent. during asphyxiation [169]), and this increase is associated with the absorption of more fluid from the endings so that the

air extends into finer branches of the tracheoles, to retreat again when the muscles rest and the metabolites responsible for the increased osmotic pressure are oxidized and removed (Figs. 165, 166).

DEVELOPMENT OF THE TRACHEAL SYSTEM

During embryonic development the tracheae arise as more or less solid ingrowths of cells from the ectoderm, enclosing between them only a potential lumen.[158] The ingrowths from adjacent segments divide and unite to produce the longitudinal trunks. Stems from these grow deeply into the tissues dividing as they go, and a lumen appears, at first in the outer trunks, then extending inwards and gradually increasing in size. The cuticular intima is then laid down and becomes thrown into spiral folds. These folds may overlie many separate cells without showing any break in their continuity; they probably arise spontaneously by the action of some simple physical force, perhaps the expansion or contraction of the previously uniform membrane, rather than by the directing influence of the epithelial cells themselves.[104, 139] The spiral folding certainly appears before any air has entered the tracheae.[158]

Development of tracheoles—At the terminations of the tracheae, certain cells separate from the general tracheal epithelium and grow out as finger-like processes towards the tissues. It is in these cells that the tracheoles are laid down as fine intracellular canals.[116, 145, 158] They may be seen penetrating the fully developed muscle fibres, and there giving rise to the intracellular tracheoles [4, 116]; and it seems probable that the intracellular tracheoles of other tissues arise in the same way, by the ingrowth of the tracheal cells.[151]

Appearance of air in the tracheal system—The tracheal system is completely formed before any air appears in it. About the time of hatching from the egg the fluid, which until then fills the lumen of the tubes, is absorbed into the tissues.[35, 71, 158] Sometimes this does not happen until the spiracles are exposed to the air, either within the shell, as in the flea or mealworm,[132] or after hatching, as in the Hemiptera or Anoplura.[132] But often, both in aquatic insects such as Chironomids [71] or Odonata [146] in which the tracheal system is closed, and in Muscids [158] and Lepidoptera [132] in which the system is later open to the air, the tracheae fill with gas while the insect is still bathed in fluid. In the one case the air enters from outside, in the other it is liberated from solution in the tissue fluids.

In the larva of the mosquito *Aëdes aegypti* the fluid is absorbed from the tracheal system by the secretory activity of the cells bounding it (Fig. 167). This larva is able to defer absorption for several days after hatching, if it is not permitted to expose its spiracles at the water surface; in fact, the nervous system seems to be responsible for initiating the absorptive activity of the tracheal walls.[170] These observations make it probable that the filling of the tracheal system in other insects also is under the control of the cells. But it is equally clear that very powerful physical forces (osmotic pressure or imbibition perhaps) are employed; forces sufficient to lead to the rupture of the continuous column of liquid in the closed tracheal system.[71, 132]

At each moult a similar process takes place. The space between the old tracheal cuticle and the new becomes filled with fluid continuous with the

moulting fluid of the body surface. In the larvae of Hymenoptera and Lepidoptera an exuvial gland like those of the body surface (p. 25) opens into the tracheae near the spiracle and is perhaps concerned in producing part of the moulting fluid.[129] As soon as moulting has taken place and the

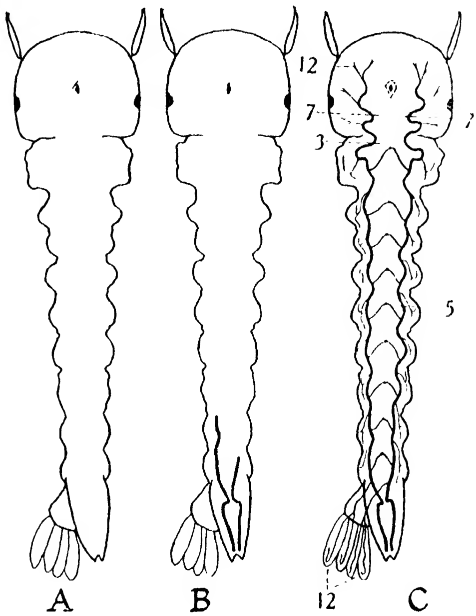

FIG. 167.—Entry of air into the tracheal system of the mosquito larva *Aëdes aegypti* after hatching from the egg (*after* WIGGLESWORTH)

A, before entry of air ; B, system partially filled ; C, system completely filled. The numbers in C show the time in minutes after exposure of the spiracles to the air ; they mark the level to which the columns of air extended at these times.

old linings of the tracheae have been drawn out through the spiracles, the fluid is actively absorbed by the cells forming the new tracheal wall.[170, 173] This is one of the many co-ordinated changes which take place at moulting.

THE TRANSPORT OF OXYGEN TO THE TRACHEAL ENDINGS

Site of oxygen uptake—It seems probable that in most insects, under ordinary conditions, comparatively little respiration takes place through the skin.[52] The cockroach, *Blatta*, will remain alive many hours with the spiracles blocked with paraffin.[122] But it will survive similarly in the complete absence of oxygen (p. 343). On anatomical grounds it is probable that most of the oxygen taken up enters the tissues through the walls of the tracheoles ; for these are always most abundant in such organs as the wing muscles, ovaries, &c., with high oxygen requirements. And if reduced indigo (indigo white) is injected into the body of a living insect, it is oxidized to indigo blue almost solely around the terminal network of tracheoles.[98, 124] On the other hand, the tracheal walls are permeable to gases,[34, 53, 165] and it is impossible to say how much of the oxygen consumed passes through them.

Mechanism of oxygen absorption—It is generally supposed that passage

of oxygen from the tracheoles into the tissues takes place by physical diffusion. But several authors have suggested that the tracheal epithelium, and particularly the tracheal end cells, play a more active part.[6, 177] In many insects (Orthoptera, Odonata) the cellular matrix of the tracheae is filled with granules of pigment; these show reversible colour changes on treatment with reducing agents [78] and have been believed (though without experimental evidence) to play a part in furthering oxidations or in storing up oxygen.[126] Similarly, the tracheal end cells, which are very active in oxidizing injected indigo white, and in reducing osmic acid when the living insect has been exposed to this vapour, have also been credited with important functions in respiration.[96, 124]

Diffusion theory—But it is certain that at least the greater part of the uptake of oxygen occurs in the tracheoles; and the main problem in insect respiration is the supply of oxygen to these endings. It was assumed long ago by Treviranus and by Thomas Graham (1833) that many insects must be dependent on diffusion; but this hypothesis was not generally accepted until Krogh,[86] taking into consideration the average diameter and length of the tracheae, the oxygen consumption of the insect and the diffusion constant of oxygen, showed by calculation that diffusion alone would supply the tissues with those quantities of oxygen actually consumed, and yet maintain at the commencement of the tracheoles a partial pressure of oxygen not more than 2 or 3 per cent. below that in the atmosphere. These calculations were made on large caterpillars which show no respiratory movements, it being assumed that diffusion takes place from the spiracles; in the case of *Dytiscus* larvae, diffusion was considered to take place from the periphery of the great flattened tracheae which are ventilated mechanically.

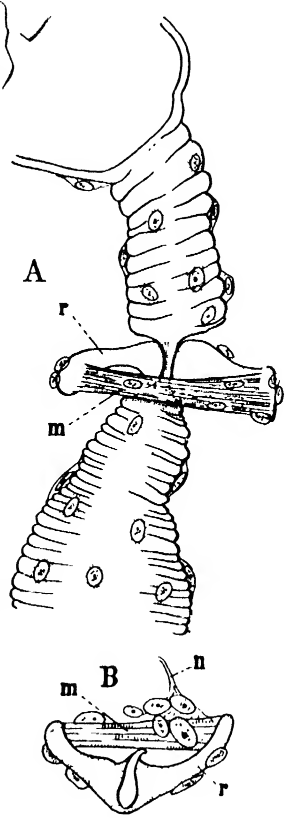

FIG. 168

A, closing mechanism on trachea of flea (*cf.* Fig. 169); *B*, transverse section through tracheae at level of closing mechanism (*after* WIGGLESWORTH). *m*, muscle; *n*, nerve; *r*, flexible rod compressing trachea.

In these calculations it was assumed also that the spiracles were open all the time. But the spiracles are almost always provided with sphincters (Fig. 168), the chief function of which is to protect the insect from loss of water. For, as in other terrestrial animals, those conditions which favour the supply of oxygen, favour also the loss of water; therefore, during rest, the spiracles of insects are kept closed most of the time, being opened only just enough to supply the insect with sufficient oxygen.[62] Hence the average tension of oxygen at the tracheal endings is probably lower than that calculated by Krogh.

'Diffusion control'—The control of respiration by the opening and closing of the spiracles is termed 'diffusion control'.[62] Its importance in regulating respiration is best seen in a small insect like the flea, in which there is no mechanical ventilation of the tracheal system.[168] In the flea (*Xenopsylla*)

at rest the whole of respiration takes place through two pairs of spiracles, the 1st * and 8th abdominal (Fig. 169). The sphincters in these spiracles often show a rhythmical opening and closing, with periods of five or ten seconds (Fig. 170). If the flea struggles, the thoracic spiracles open, and remain open for some seconds after the muscular movements cease. During the height of digestion, and while the eggs are ripening, the 1st and 8th abdominal spiracles keep open all the time, and the remaining spiracles show a rhythmical opening and closing, or may perhaps be held permanently open also. The rhythm of all the spiracles is quickened if the temperature is raised.[168] During the period of closure of the spiracles, as the gas within the tracheal system is used up, many of the larger tracheae in the flea collapse

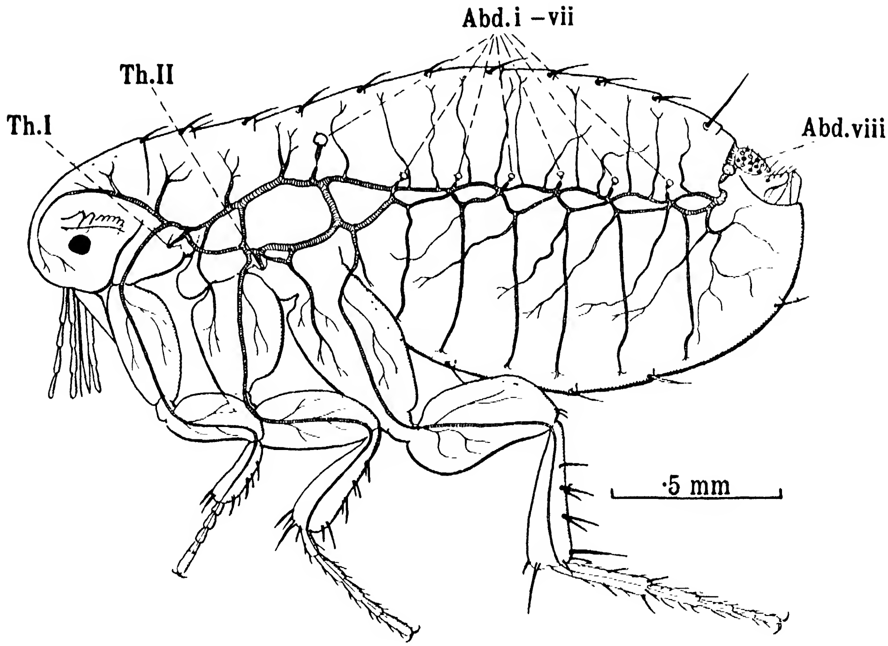

FIG. 169.—One-half of the tracheal system of the flea *Xenopsylla* (*after* WIGGLESWORTH) *Th.I*, *Th.II*, thoracic spiracles ; *Abd. i–viii*, abdominal spiracles.

and are flattened, while the smaller tracheae are compressed in the long axis.[64] The same thing is seen in larvae of mosquitoes if they remain long under water.[7]

Spiracles and loss of water—The importance of the spiracular closing mechanisms in conserving water is well seen if the evaporation of water from the living insect is measured quantitatively.[101] In one experiment, a batch of adult *Xenopsylla* were caused to double their rate of loss of water when the spiracles were kept open by exposure to 5 per cent. carbon dioxide (Fig. 171, B). One recently fed mealworm larva increased its rate of loss from 5·1 hundredths of a milligram per hour in air, to 12·5 in 5 per cent. CO_2 in air ; and after starving for four months, as the result of which its rate of

* In the original paper the author followed Lass [89] in regarding this spiracle as the 3rd thoracic. It is more likely that the flea, like other insects, has only two pairs of thoracic spiracles.[134]

metabolism was reduced so that the spiracles were normally opened less frequently, it increased its water loss from 1·5 hundredths of a milligram

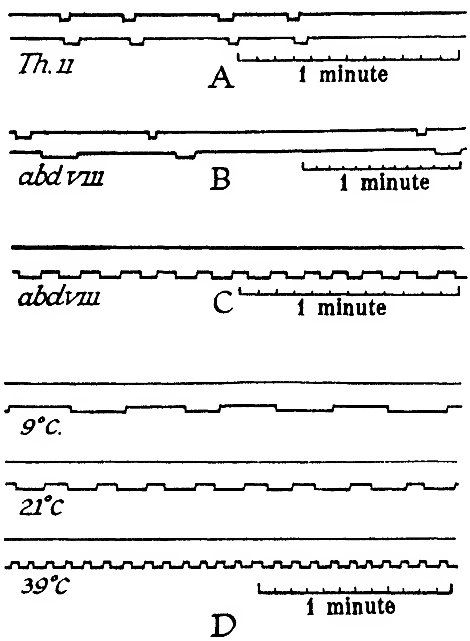

FIG. 170.—Movements of spiracles in the flea *Xenopsylla* (*after* WIGGLESWORTH)

The upper line of each tracing indicates periods of struggling; the lower line shows opening (downwards) and closing of the spiracles. A–C, at 22° C. *A*, second thoracic spiracle opening soon after struggling has begun; *B*, eighth abdominal spiracle in fasting flea opening after struggling has ceased; *C*, eighth abdominal spiracle in flea after feeding, showing rhythmical opening and closing in the absence of muscular movement; *D*, effect of temperature on the rhythmical opening and closing of the eighth abdominal spiracle.

per hour in air, to 11·1 in 5 per cent. CO_2 in air (Fig. 171, A)[101] The bug *Rhodnius*, which is normally very resistant to desiccation, dies in about 3 days

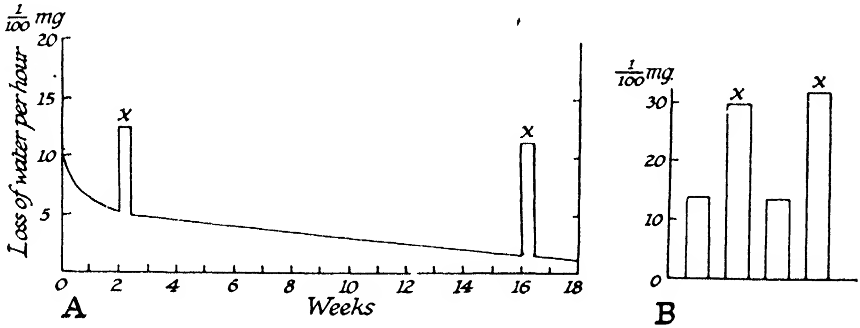

FIG. 171.—The effect of opening the spiracles on the rate of loss of water (*after* MELLANBY)

A, rate of loss in 1/100 mg. per hour from a single mealworm starved over a period of 4 months. At the point *x* the insect was caused to open the spiracles. *B*, rate of loss from a number of adult fleas (*Xenopsylla*) in 1/100 mg. per hour per mg. of insect. Columns marked *x* obtained when the spiracles were kept open with 5% carbon dioxide.

in a dry atmosphere if it is caused to keep its spiracles open.[171] And if the spiracles of a given group of beetles are compared, the xerophilous forms

accustomed to a dry environment are found to have spiracles better adapted to prevent loss of water than the hygrophilous forms; in the former the openings are often small and deeply sunk; and in xerophilous Buprestids there is a basketwork of outgrowths across the openings, an arrangement which is believed to impede the diffusion of the aggregated molecules of water more than the carbon dioxide and oxygen.[12]

'Ventilation control'—In addition to this regulation of diffusion, many insects effect a varying degree of mechanical ventilation of the larger tracheal trunks, or 'ventilation control'. It has often been suggested [7, 88, 123] that the muscular movements of the insect, and the contractions of the heart, intestine, Malpighian tubes and so forth, by pressing upon the tracheae, will assist their ventilation, and

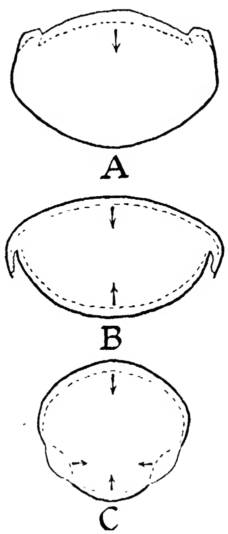

FIG. 172.—Types of expiratory movements in abdomen of insects (*from* SNODGRASS *after* PLATEAU)

A, movement almost confined to the terga (Heteroptera and Coleoptera); *B*, terga and sterna both take part in the movement (Odonata, Acrididae, Aculeate Hymenoptera, Diptera); *C*, terga and sterna separated by ample membranous areas which are drawn inwards as the terga and sterna approach (Tettigoniidae, Neuroptera, Trichoptera, Lepidoptera).

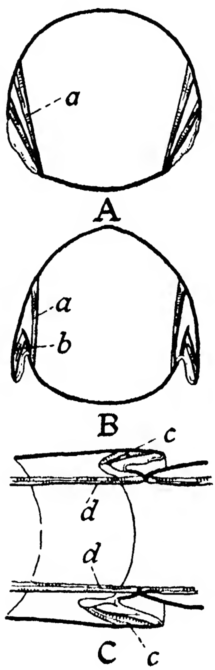

FIG. 173.—Diagrams of respiratory muscles in abdomen of insects (*after* SNODGRASS)

A, segment with compressor muscles only; *B*, with compressor and dilator muscles; *C*, longitudinal section showing protractor and contractor muscles which bring about the telescoping movements. *a*, compressor muscle; *b*, dilator; *c*, protractor; *d*, contractor.

will do so more or less in proportion to the needs of the animal. But in the large caterpillar, *Cossus*, even the most violent contractions of the body cause very little ventilation—at most 2–9 c.mm. in a total tracheal volume of 50 c.mm.[86]

In many insects, however, particularly in active adult forms, specialized movements of the body wall occur, which serve the purpose of ventilating the tracheal system. These may consist of peristaltic waves over the abdomen as in *Tipula*, dorso-ventral flattening of the abdomen in many forms: grasshoppers, beetles, &c., telescoping movements of the abdominal segments in

Hymenoptera and Diptera (Fig. 172).[6, 117, 123] In *Dytiscus* and *Hydrophilus*,[22] in *Dixippus* [25] and doubtless other insects the thorax also may take part in the movements of expansion and contraction. As a rule expiration is the active movement and takes place more brusquely than inspiration, which is often effected by the elasticity of the body wall; but in certain cases, as in the larva of *Aeschna* [155] and some grasshoppers,[91] inspiratory muscles also are present (Fig. 173). If there is a respiratory pause it is always at the end of inspiration.

The function of the respiratory movements is partially to renew the air in the tracheae by alternate compression and expansion of the tracheal system, the changes in pressure being transmitted from the body wall by the haemolymph. At no stage is there any material compression of the gas in the tracheal system.[39, 86] In virtue of their spiral folding the tubular tracheae resist compression from side to side, but they are very extensible; they can be stretched to double their length without injury [128] or can by shortened 20–30 per cent. by a pressure of 5·6 cm. of mercury.[39] And many of the larger tracheae, such as the longitudinal trunks in *Dytiscus* larva [118] or the tracheae between the flight muscles of Muscid flies, are oval in cross section and cannot resist collapse. If the ventral surface of the adult water-beetle, *Cybister*, is examined with a lens while breathing at the surface, the tracheae can be seen through the transparent integument alternately flattened and dilated.[20] In the larva of *Eristalis* the enlarged tracheal trunks show both changes during expiration; they not only flatten but become shorter, the taenidia closing up like the coils of a spring.[41]

Functions of air sacs—The ventilation of the tracheal system is further helped by the special modifications of the tracheae present in those insects which show the most pronounced respiratory movements. The innumerable thin-walled saccular dilatations which occur all along the larger tracheae in the cockchafer, *Melolontha*, are collapsed and empty during expiration, whereas the tubular tracheae themselves appear unchanged.[39] In the larva of *Eristalis*, the tracheal sacs formed as dilatations of the main trunks are rhythmically collapsed and expanded by the integumental muscles.[2] In other insects, especially those with well-developed powers of flight, similar dilatations form large air sacs which extend into all parts of the body. Even where these lie in rigid parts such as the head or legs, the changes in pressure produced by the respiratory movements of the abdomen will be transmitted to them by the blood.[30, 57, 92]

1

There can be little doubt, therefore, that the chief function of the air sacs is to increase the volume of the 'tidal air' which is renewed at each respiration. But this is certainly not their only function. As pointed out by John Hunter [110] they also serve to lower the specific gravity of the insect (the massive head and mandibles of such beetles as *Lucanus cervus* are largely filled with tracheal air sacs) and to that extent they will assist in flight. But their supposed buoyant action as the result of the air within them being heated by muscular contractions is certainly not significant. In the abdomen of Muscid flies (*Calliphora*, &c.) there is a pair of vast air sacs, the main function of which seems to be simply to provide a space into which the abdominal organs can grow without influencing the outward form of the abdomen. They are fully distended in the immature female fly; as the ovaries ripen

and the fat body enlarges they become collapsed without interfering with respiration (Fig. 174).[47, 51]

It is often said that the air sacs serve as reservoirs for oxygen during flight. But in most insects studied, *Vespa*, *Schistocerca*, Sphingids, respiratory movements of great amplitude take place all the time the insect flies.[50] It is true that in certain beetles, *Cetonia*, *Melolontha*, the respiratory movements are arrested at least during short flights [50]; the explanation of this is not known; but the spiracles are said to remain open [26] and it is possible that the tracheae are being ventilated by the direct action of the wing muscles.[123]

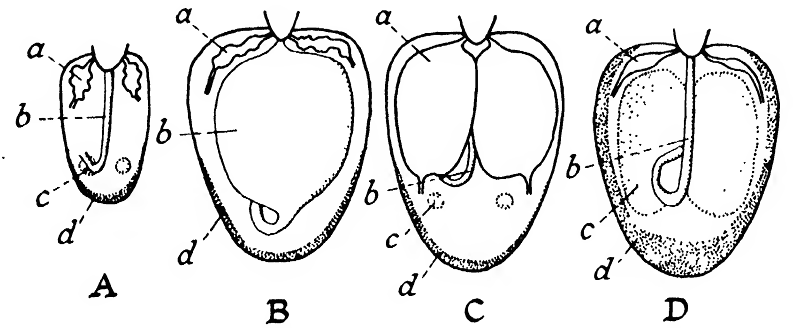

FIG. 174.—Diagram showing the part played by the great air sacs in the abdomen of *Lucilia* during the growth changes in the adult fly (*after* EVANS)

A, fly just emerged. *B*, after 5 minutes; gut filled with air, abdomen distended. *C*, after 10 hours; gut collapsed, air sacs distended. *D*, fully fed for 6 days; ovaries and fat body fill abdomen, air sacs collapsed again. *a*, air sacs; *b*, gut; *c*, ovaries; *d*, fat body.

Efficiency of tracheal ventilation—Some indication of the efficiency of the mechanical ventilation of the tracheal system is given by measurements of the respiration volume or tidal air in relation to the total capacity of the system. In the larva of *Dytiscus* the total capacity of the tracheal system is some 107 c.mm. (6–10 per cent. of the total volume of the animal); the vital capacity is 64 c.mm. In other words, during strong expiration the tracheal system is emptied of nearly two-thirds of its total capacity.[86] In the adult cockchafer, *Melolontha*, with a total capacity of 630 c.mm., or about 39 per cent. of the body volume, the vital capacity is about 210 c.mm. or one-third of the total capacity.[39] The tidal air, the volume actually inspired or expired at each respiration, varies within wide limits in both these insects. It has been calculated that in the grasshopper each deep respiration will effect a 20 per cent. renewal of the air in the hind leg.[84] For the sake of comparison with pulmonary respiration, it may be recalled that the vital capacity, the extreme degree of ventilation possible, in man is about two-thirds of the total capacity; in normal quiet respiration the tidal air is very much less than this.

Streams of air through the tracheal system—In some insects the mechanical ventilation of the tracheal system doubtless takes place through all the spiracles indiscriminately. But in many forms the opening and closing of the spiracles in different parts of the body is so timed in relation to the pumping movements that inspiration occurs predominantly in one region, expiration in another; with the result that there is a more or less directed flow of air through the tracheal system. If the 4 anterior spiracles in *Schis-*

tocerca are separated by a diaphragm from the 6 posterior pairs, there is a transference of air from the anterior half to the posterior varying from 5–20 c.mm. per second (Fig. 175).[60] In another grasshopper, *Chortophaga*, the stream also enters chiefly by the thorax and leaves by the abdomen[91]; at 28° C. an average of about 2·5 c.mm. and a maximum of 12·5 c.mm. are passed through per second.[100] But only part of the movement is a through movement; for if the carbon dioxide given off from the two parts is compared, 20 per cent. comes from the thorax and 80 per cent. from the abdomen[100]; and the rhythm of spiracle movements and the direction of flow may change from time to time.[99] When the respiration of *Chortophaga* is strongly stimulated by 15 per cent. carbon dioxide, the flow may be reversed or the passage of air may cease.[100] In the large cockroach, *Nyctobora*, the normal flow is in the same direction and in similar quantities; when increased by carbon dioxide the flow is maintained against a pressure of 20 cm. of water.[73]

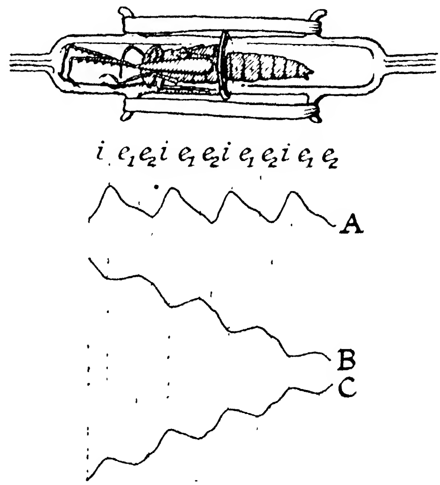

Fig. 175

Above, apparatus for demonstrating the directed stream of air through the tracheal system; grasshopper with thorax and abdomen in two halves of gas chamber separated by rubber partition. Below, volume changes in the apparatus during normal respiration; schematic. *A*, respiratory movements; *B*, fall in volume in the thoracic half of gas chamber; *C*, increase in volume in abdominal half. *i*, inspiratory phase; thoracic spiracles opened, abdominal spiracles closed; e_1, first part of expiratory phase, all spiracles closed; e_2, second part of expiratory phase, abdominal spiracles open; air streams out into posterior part of chamber. (*After* Fraenkel.)

In many aquatic insects, although on occasion any of the spiracles can be used either for inspiration or expiration, the structure and balance of the body is such that in the normal position of respiration at the water surface some spiracles are more important in inspiration than others.[14, 68] In *Hydrophilus*, which comes head first to the surface, inspiration is believed to occur chiefly by the mesothoracic spiracles and expiration by the abdominal spiracles; in *Dytiscus*, which rises tail first, the terminal spiracles of the abdomen are chiefly used in inspiration, and so on.[20]

But in all these insects, whether the respiratory movements cause a simple tidal flow in and out of the spiracles, or whether they bring about a directed stream of air through the system, they can ventilate only the larger tracheal trunks. The finer branches must always be dependent on diffusion.

THE ELIMINATION OF CARBON DIOXIDE

We have seen that diffusion through the smaller tracheal branches, combined in many insects with mechanical ventilation of the larger trunks, will explain the supply of oxygen to the tissues through the tracheal system. Since the volume of carbon dioxide produced in metabolism is generally somewhat less than the oxygen consumed, and its rate of diffusion through air is not much slower, there is no difficulty in accepting the elimination of carbon

dioxide by the same route, particularly as the partial pressure of carbon dioxide in the surrounding atmosphere is almost zero. But carbon dioxide diffuses through animal tissues very much more readily than oxygen; it will diffuse through the connective tissue of vertebrates nearly thirty-five times as rapidly.[85] Although exact measurements have not been made, it is probable that the same applies to the cuticular coverings of insects. It is therefore almost certain that both the body surface and the linings of the tracheae play a much larger part in the elimination of carbon dioxide than they do in the uptake of oxygen.

This belief is supported by several observations. In the soft-skinned larvae of beetles and other insects, carbon dioxide is easily demonstrated escaping from the general body surface; in the heavily schlerotized adult Coleoptera, this applies only to the intersegmental membranes.[140] In the endoparasitic Agromyzid larva, *Cryptochaetum*, whereas the uptake of oxygen occurs most actively in the respiratory tails, the elimination of carbon dioxide takes place more or less equally over the general body surface.[141] From measurements of the output of carbon.dioxide from insects or parts of insects in which the spiracles were occluded, it has been estimated that in the stick insect *Dixippus*, about 25 per cent. of the total output escapes through the skin; in the larvae of *Dytiscus* and *Eristalis* somewhat less.[25] The absorption of oxygen through the skin in these insects is certainly far less than that.

It has often been observed that the greater part of the carbon dioxide is given off by the posterior half of the body. In some cases this is due to the backward circulation of air through the tracheal system—as in the locust *Chortophaga*, in which 80 per cent. comes from the abdomen.[100] Where it happens in insects without respiratory movements (in the large caterpillar, *Harpyia*, nearly 75 per cent. comes from the posterior half) it may be due to the carbon dioxide carried backwards by the circulating blood being eliminated by the rich tracheal network connected with the last two pairs of spiracles[176]; or it may be due simply to the fact that in quiet respiration certain spiracles alone are functional—in the flea the 8th abdominal spiracles.[168]

THE RESPIRATION OF AQUATIC INSECTS

The structural adaptations of aquatic insects for respiration are immensely varied,[36, 102, 118] but here we shall deal only with examples which serve to illustrate the problems involved and the principles upon which their solution has been based.

Cutaneous respiration—The most complete adaptation to life in water is shown by certain larvae in which the tracheal system, though developed in the usual way by ectodermal invaginations, has become entirely cut off or 'closed' from the exterior by the obliteration of the stigmatic branches.[112] During the early stages of some larvae of this type, *Chironomus*,[115] *Simulium*,[153] *Acentropus* (Lep.),[111] the system may remain filled with fluid; and then respiration must be accomplished by a simple diffusion of gases through the skin. In a few insects, for example the aquatic Hymenopteron *Polynema*,[65] the tracheal system may remain entirely devoid of air even in the adult; but as a rule, after the first instar, if not sooner, the system contains gas.

So-called 'blood gills'—In some larvae certain regions of the integument

are often exceedingly thin and project from the body surface (*Chironomus*), or are evaginated from the rectum (*Simulium*), as delicate blood-filled sacs, devoid of tracheae or provided with only a few small branches. These are often termed 'blood gills', through which it is supposed that the gaseous exchanges chiefly occur.[103, 153] But papillae of this type exist equally in larvae, such as *Eristalis* and other Syrphids, Culicids, &c., which breathe atmospheric air at the water surface; and what experimental evidence exists is against their having any respiratory function. Various protozoa (*Bodo*, *Polytoma*, &c.) which form aggregations at a particular concentration of oxygen in the water [46] have been used as indicators for this purpose. When the larva is immersed in a culture of such organisms they first congregate at those points where oxygen is being most actively absorbed or consumed. Then, as the oxygen concentration falls below the optimum, they form a band or shell which stands out farther from such points than elsewhere on the body surface (Fig. 176). Experiments on these lines have shown that the anal and ventral papillae of *Chironomus*,[49] the anal papillae of mosquito larvae [167] and the rectal blood gills of Trichoptera, *Simulium*, *Corethra*, &c.,[142] are not the site of particularly active oxygen uptake, though it has recently been suggested that the ventral gills of *Chironomus* may be of value during recovery from oxygen want.[61] Moreover, the rectal papillae of *Eristalis* are not extruded more frequently during asphyxiation.[2] We shall consider the function of these organs in a later chapter (p. 283).

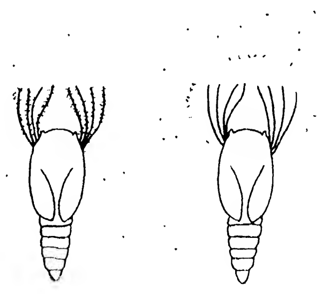

FIG. 176.—Respiratory function of the cuticular gills in *Simulium* pupae demonstrated by means of the flagellate *Bodo* (*after* Fox)

A, soon after immersion in the culture; flagellates collecting on the surface of the gills. *B*, later; flagellates concentrated in a band some distance from the gills.

Tracheal gills—On the other hand there are many modifications of the closed tracheal system undoubtedly directed to obtaining oxygen from the water. The skin is often supplied with a rich network of fine capillaries, as in *Simulium*,[102] *Chironomus*, [177] *Acentropus*,[111] or the 1st instar of *Corixa*.[59] This network may be most abundant where the cuticle is thin, as in Trichoptera [95] or Plecoptera,[130] or in the larva of the Ceratopogonid *Atrichopogon*, which has a series of richly tracheated cushions along the back.[27] Then, in various regions of the body wall, or within the rectum, there may be evaginations well supplied with tracheae which form true 'tracheal gills' [36, 112]; as in Ephemeridae,[149], Trichoptera,[95] Plecoptera [90, 94], and Odonata.[125, 146]

But cutaneous respiration is still important in most aquatic larvae even when efficient tracheal gills are present.[143] The same applies of course to other animals; a large part of the normal respiration of frogs takes place through the skin and at low temperatures they can live without the lungs. It is this fact which has led to much controversy as to the importance of the tracheal gills in respiration. The sixty tracheal gills of the caddis larva (*Macronema*) can be removed and cause almost no reduction in the oxygen consumption.[107] The lateral gill plates of Ephemerid nymphs can be removed without causing the

death of the insect ; cutaneous respiration by itself is sufficient in winter, during rest and in highly oxygenated water ; but the gills become indispensable in summer, during activity and in water poor in oxygen.[32, 106] The gill plates of these nymphs are moved more vigorously in water that is poor in oxygen [9] and when different species are compared there is found to be an inverse correlation between gill area and the oxygen content of the water in which they live.[40] In normal nymphs of *Cloëon* at 10° C. the rate of oxygen uptake does not begin to fall off steeply until the oxygen in the water has fallen to 1·5 c.c. per litre, whereas in gill-less nymphs under the same conditions this occurs at about 2 c.c. per litre (Fig. 177). At these low concentrations the gills beat actively; but still some doubt exists whether the gaseous exchange is occurring through the gill plate itself, or whether the gills are merely irrigating the true respiratory surfaces.[172] Similarly, it has long been known [57, 166] that Agrionid nymphs can survive without the caudal lamellae ; yet it has been estimated from measurements of the gaseous diffusion into the tracheal system of normal and gill-less nymphs, that in *Agrion pulchellum* 32–45 per cent. of the oxygen uptake normally enters by the lamellae.[77]

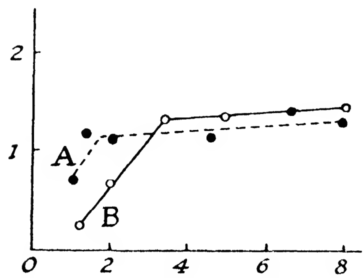

FIG. 177.—Oxygen uptake in nymphs of *Cloëon dipterum*

A, normal insects ; *B*, insects with gills removed. Ordinate : oxygen consumption (c.c./gm./hr.). Abscissa : oxygen concentration (c.c./litre). (*After* WINGFIELD.)

The closed tracheal system—The mechanism by which the exchange of gases in the closed tracheal system is effected has been studied only in the nymphs of Anisopterid dragon-flies. These have an elaborate system of tracheal gills, the branchial basket, consisting of six double rows of imbricated lamellae, situated in the rectum ; they breathe by passing water in and out of this gill chamber.[128] Sometimes the water is 'swallowed' in small quantities, and mixed by peristaltic movements [147] ; but in an actively respiring mature nymph of *Aeschna*, each pumping movement of the abdominal wall will effect about 83 per cent. renewal of the contents of the hind-gut, the ventilation volume per minute amounting to about 1 c.c.[155]

The gaseous exchange in these dragon-fly nymphs was attributed by Dutrochet (1837) to simple diffusion ; and that view is now pretty generally accepted. A closed tracheal system can only function on this plan provided it has non-compressible walls ; and in fact it has been shown that in *Aeschna* a pressure increase of half an atmosphere causes at most a compression of 4–5 c.mm.[86] ; although the spiracles are open, their structure resists the entry of water : even at a pressure of 10 metres of water none passes in.[78] The gills themselves are well suited to maintain a diffusion equilibrium between the water and the tracheal gases, the membrane being very thin and the tracheoles lying immediately below it (Fig. 178). And it has been shown quantitatively that oxygen diffuses with equal rapidity in the two directions : the membrane behaves like a dead membrane, complying with the laws of diffusion.[78] Moreover, in the living nymph the partial pressure of oxygen in the system is always less than the tension of oxygen in the water : 10 per cent. of an atmosphere in the water, 5–6 per cent. in the tracheae ; 35 per cent. in the water, 20–30 per

cent. in the tracheae.[155] We have seen that if the water pressure is increased, none enters the spiracles ; but these do not resist the outward passage of air. If the larva is placed in alcohol and water, in which the solubility of air is reduced, bubbles of gas immediately escape from one or more of the spiracles.

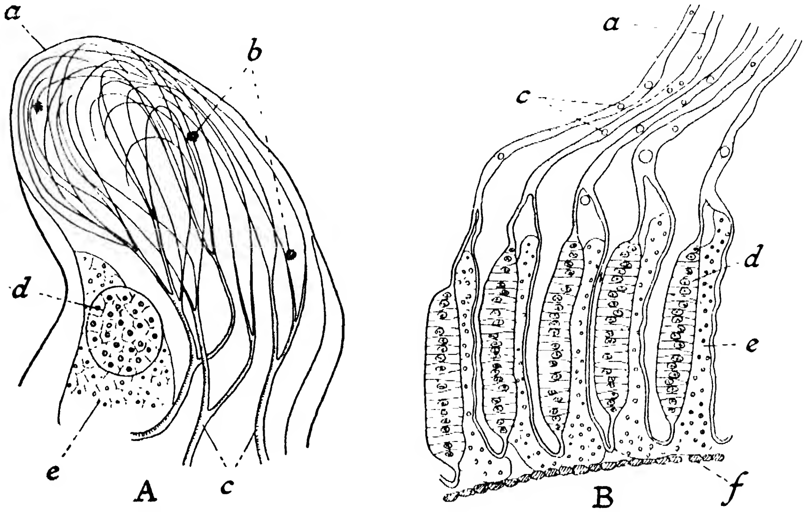

FIG. 178.—Gill lamellae in the rectum of *Aeschna* nymph (*after* SADONES)

A, surface view of lamella ; *B*, longitudinal section through series of lamellae. *a*, gill plate ; *b*, tubercles ; *c*, tracheae and tracheoles ; *d*, epithelial pad at base of gill plate ; *e*, fat body ; *f*, circular muscles.

This is because the pressure of gas in the tracheal system now exceeds the pressure of the dissolved gases ; it affords confirmatory evidence that the exchanges are normally effected by diffusion.[78]

Cuticular gills—There is another type of gill in aquatic insects in which there can be no question of any secretory activity. These are termed ' tube gills ' or ' cuticular gills ' ; they occur in the larva of *Teichomyza* (Dipt.),[52] and in the pupae of *Simulium*,[138] *Deuterophlebia* and probably the Blepharoceridae [121] ; and perhaps the branched rosettes of hollow tubes containing air, which replace the spiracles of the Hemipteron *Aphelochirus*,[137] are of the same type. They arise as filamentous outgrowths of the ectoderm and are, therefore, covered with a layer of cuticle. The lumen of the filament loses all connexion with the body cavity, and the epithelium more or less degenerates ; but the cuticular wall becomes much thickened and is excavated to form air spaces which establish a connexion with the tracheal system. Thus the air in the tracheae is separated from the water only by a delicate wall of cuticle about $0{\cdot}1\mu$ thick. The pupae of *Simulium* can develop after removal from water ; this type of gill is probably an adaptation to life in mountain streams which are liable to dry up.[121]

Surface breathing ; hydrofuge structures—So far we have considered only aquatic insects which breathe dissolved oxygen ; but the great majority of aquatic forms are dependent on the atmospheric air. These show many structural adaptations which facilitate the connexion of certain spiracles with the atmosphere, such as the terminal respiratory tube of *Nepa*, the modified antennae of *Hydrophilus* and its allies which come head first to the surface, the

balance of the body in Dytiscids which rise tail first, the respiratory siphons of *Eristalis*, Culicids, &c., bearing the spiracles at the apex. These we cannot discuss here in detail. But a problem common to all these insects is the breaking of the tough ' surface film ' of the water so that the spiracles may be exposed to the air above. This problem has been solved in all cases by the same device; by the provision of regions of the body wall whose physical properties are such that they have a greater affinity for air than for water, which are in fact ' hydrofuge '—the ' angle of contact ' between the water and the region in question being relatively large.[10] The cohesion of the water is then greater than the adhesion to the body; when the insect reaches the surface film, the water therefore falls away and leaves the cuticle dry. In order to furnish this property in the larvae of Diptera (*Eristalis*,[41, 154] Tachinids,[113] Culicids, &c.[72]) there are always small glands at the spiracular openings (perispiracular glands) which produce an oily secretion; and in *Dytiscus* similar glands probably occur all over the body surface.[15]

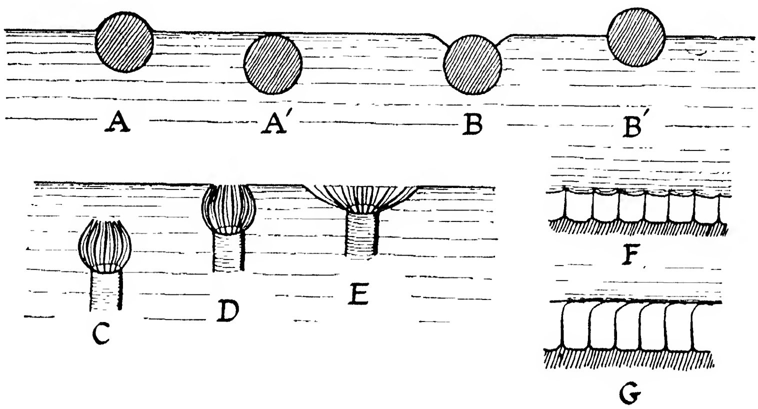

FIG. 179

A, A′, hydrophile hair in the water surface seen in cross-section; *A′* is the position of equilibrium. *B, B′*, hydrophobe hair in the water surface; *B′* is the position of equilibrium. *C*, spiracle surrounded by crown of hydrophobe hairs (as in *Stratiomys* larva); this is the position of equilibrium below the surface. *D, E*, the same at the water surface; *E* shows the position of equilibrium. *F*, short hydrophobe hairs standing erect on the cuticle (as on ventral surface of *Dytiscus*). *G*, hydrophobe hairs curved over at the tip (as in *Elmis* and *Harmonia*).

Another device consists in the provision of a fringe or crown of long hairs (around the spiracles of *Notonecta* [20, 68] or along the antennae of *Hydrophilus*) which are held in the surface film as though they were hydrophile on one side and hydrofuge on the other. On reaching the water surface a fringe of this kind is spread outwards by surface tension, and the air space within put into communication with the atmosphere. The physical explanation of these ' semi-hydrofuge ' hairs is given in Fig. 179, which shows cross-sections of hairs of different types placed in the water surface. In Fig. 179, A, the hair is hydrophile with an angle of contact approaching zero. In such a case the free energy of the surface is at a minimum, that is, the surface area is smallest, when the hair is completely wetted (Fig. 179, A). In Fig. 179, B, the hair is hydrophobe, the angle of contact being 90°. Here the surface energy is least when the hair is held in the water surface with half in air and half in water; hence that is the position of equilibrium. When below the surface, the boundary between air and water will be smallest when the crown of hairs is drawn together

(Fig. 179, C); on exposure to the air, the interface will be smallest when the fringe is widely spread (Fig. 179, E).

These hydrofuge structures around the spiracles also have the function of preventing water from entering the tracheal system. The protective action of the spiracles of the larva of *Gastrophilus* is destroyed by bile in a dilution of 1 per cent., and the tracheae fill with fluid. But while water cannot get in, oil can do so readily, and spreads throughout the tracheal system. It is this property which lies at the back of the observation made by Aristotle, and explained by Malpighi, that oil is always fatal when applied to the surface of insects; and it is this property which forms the basis of the classical method of controlling mosquito larvae by spraying oil on the surface of the water.[166]

Air stores of aquatic insects—Besides retaining a film of air immediately around the spiracles, many aquatic insects are able to carry bubbles or films of air on other parts of the body. In the larva of *Hydrocampa* (Lep.),[118] and on the ventral surface of *Dytiscus*, *Notonecta* and many other insects, there is a fine pile of erect hydrofuge hairs, which hold between them a layer of air, like velvet when immersed in water (Fig. 179, F). In the aquatic Coleoptera *Harmonia* and *Elmis*, there are long 'semi-hydrofuge' hairs all over the surface of the body which are bent over at the tips; hence, as explained above, the outer surface of the free ends appears hydrophile, and so provides a hydrophile surface enclosing beneath it a thin film of air (Fig. 179, G).[20] Dytiscids carry considerable quantities of air beneath the elytra, or as a bubble attached to the posterior extremity. We must now consider the function of such air stores.

In the first place these stores of air have an important hydrostatic function in enabling the insect to rise to the water surface, and to do so in the correct position for renewing its supply of oxygen. If weights of different magnitude are attached to *Notonecta*, the diminution in buoyancy which this causes is compensated by an increase in the volume of air carried in the air store.[17] Indeed some writers have regarded the air stores of *Dytiscus*, [20, 22, 162] *Notonecta*, *Corixa*, *Nepa*, *Hydrophilus* [20] as containing only expired air which is normally of no further value in respiration.

That, however, is an overstatement of the case; the air in the stores can certainly be breathed in again through the spiracles,[20] and to this extent they constitute an oxygen reserve. Thus in *Dytiscus*, the respiratory pumping movements continue after submergence,[8] and the oxygen content of the elytral air falls rapidly from 19·5 per cent. at the moment of diving, to 1 per cent. or less in three or four minutes.[44, 82] If the capacity of the subelytral space is increased by removing the hind wings, the average duration of submergence is prolonged.[63]

But the air stores have a third function, which consists in separating by diffusion the oxygen dissolved in the water. This function has been suggested by many authors for a great many different insects [166]; but the most instructive paper from a theoretical and quantitative standpoint is by Ege,[44] who shows that the capacity of such a film of air to function as a 'gill' in this way depends on the fact that the invasion coefficient of oxygen between water and air is more than three times as great as that of nitrogen. Consequently, if the tension of oxygen in the water is higher than the partial pressure of oxygen in the air film, there will be a greater tendency for equilibrium to be restored by the diffusion of oxygen into the bubble, than by the diffusion of nitrogen out of it.

During summer, when the insects are actively swimming, they cannot obtain enough oxygen by diffusion from the water in this way; but the mechanism is of more or less value in all aquatic insects, which will benefit to the same extent as though they were provided with about 13 times as much air as they actually carry. *Corixa* can obtain enough oxygen in this way even at summer temperature so long as it does not swim actively; and this applies even to the much larger *Dytiscus* during the winter. To quote a single experiment showing the value of the process: *Notonecta* lived 7 hours in water saturated with atmospheric air, 35 minutes in water saturated with oxygen, and 5 minutes in water saturated with nitrogen. Thus, in the second case, although the insect had five times as much oxygen in its store as in the first case, it survived only one-tenth of the time, because, in the absence of nitrogen, invasion of the air store by oxygen could not take place. Of course the process cannot continue indefinitely because the nitrogen in the air store is gradually dissolved; and if the insect is compelled to remain submerged, all the gas in its store eventually disappears into solution. In fact, with the smaller insects, in which the invasion process plays a more significant part, it is really of more importance for the insect to renew its store of nitrogen than to obtain a fresh supply of oxygen.

In a few cases, for example in the beetles *Harmonia* and *Elmis* [20] (see p. 201), the air film is so held by the hydrofuge hairs than it cannot be replaced by water. Conditions then approximate to the closed tracheal system, and the insect becomes independent of the atmospheric air. *Elmis*, for instance, will live for months below the surface, if the water is well aerated.

Aquatic plants as source of oxygen—There is yet another way in which aquatic insects have become independent of the water surface. This is by obtaining their oxygen from aquatic plants. The free bubbles of gas given off by plants are frequently taken up by the hydrofuge surfaces of insects [20, 173]; and with the approach of winter many aquatic insects congregate in those pools which are most rich in vegetation.[162] Other insects obtain their oxygen from the intercellular air spaces of water plants, either by biting into the air-containing tissues, as in *Elmis* (Col.) [20] and the pupating larva of *Hydrocampa* (Lep.),[45] or by inserting a specially modified respiratory siphon into the air-containing cells. Different modifications for piercing plants and obtaining oxygen in this way have been evolved independently by larvae in two families of Coleoptera and three families of Diptera.[148] The best known examples are the larva of the beetle *Donacia*, first described by v. Siebold in 1859,[37] and the larva of the mosquito *Mansonia*. The oxygen content of the gas in the rhizomes and roots from which *Donacia* gets its air, seldom contains more than 10 per cent. of oxygen, and in winter it may fall below 1 per cent.[44]; though values as high as 45 per cent. have occasionally been found in *Potamogeton*.[45]

Cutaneous respiration—In all these aquatic insects which breathe by open spiracles, as in those which breathe by gills, the skin is more or less important in respiration; and the part it plays is naturally greater if the insect is obliged to remain submerged. Some species of mosquito larvae can develop completely in well aerated water without access to the surface or even if the tracheal system is filled with a non-toxic oil.[167] When the Tipulid larva *Pedicia* has its spiracular process in connexion with the air, the uptake of oxygen by the skin is very slight, but it at once becomes greatly increased if the larva is denied access to the air, the exchange being greater in the region of the 'gills'; though

this larva is said to be unable to survive permanently the occlusion of the spiracles.[143]

Hydrostatic function of tracheal system—In addition to its respiratory function the closed tracheal system has a hydrostatic function [112]; and in a few cases, notably the Nematocerous larvae *Mochlonyx* and *Corethra*, the latter function is the chief one. In *Corethra* the tracheal system is practically reduced to two pairs of bean-shaped sacs with a number of contractile pigment cells spread over the surface (Fig. 180). The gases in these air bladders come into diffusion equilibrium with any gas mixture in the surrounding water.[83] But they can vary in size (shrinking to 90 per cent. and expanding to 122 per cent. of their normal volume [34]) so that the buoyancy of the larva accords with the density and pressure of its environment, and it is enabled to float at any desired level. The substance of the wall of the sac seems to differ from that of most tracheae in being able to imbibe water; it is described as consisting of a colloid material ('trachein') which contracts on drying and swells up instantly on moistening with the insect's blood or with solutions of sodium chloride.[29, 53, 74] The living insect is able to control its buoyancy apparently by inducing an active swelling or shrinkage of these cuticular walls. The chemical mechanism of this change is not known, nor is the source of the sensory impulses by which it is evoked.[34, 53]

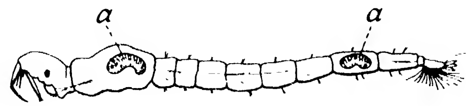

FIG. 180.—Larva of *Corethra*, showing the tracheal system reduced to two pairs of kidney-shaped air sacs (a) covered with pigment cells

RESPIRATION OF ENDOPARASITIC INSECTS

The respiration of endoparasitic insect larvae shows some striking parallels with the respiration of aquatic forms. Thus, in the 1st instar of many hymenopterous parasites,[131] and in the Agromyzid (Dipt.) parasite *Cryptochaetum*,[141] the tracheae are filled with fluid and not functional; the exchange of gases takes place directly between the tissue fluids of parasite and host. When the tracheae fill with gas, usually in the 2nd instar, they supply a rich network of fine branches to the skin (Fig. 181), and it is not until the larva is about to quit its host that the spiracles become open and functional.[131]

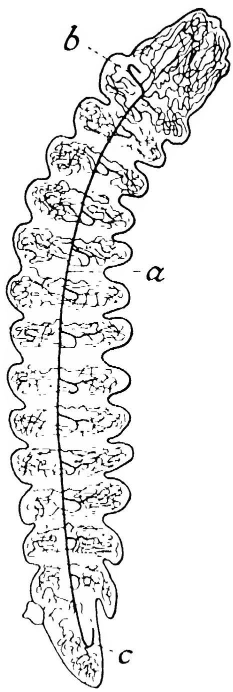

FIG. 181.—First-stage larva of *Macrocentrus gifuensis* (Ichneumonidae), lateral view, to show the rich tracheal supply beneath the skin (*after* PARKER)

a, lateral tracheal trunk; b, anterior commissure; c, posterior commissure.

'Blood gills' and 'tracheal gills'—Many Braconid, Ichneumonid and Chalcid larvae possess a 'tail' of varied form, usually best developed in the

early larval stages, which recalls the 'blood gills' of aquatic larvae. This structure has had locomotor, absorptive and excretory functions assigned to

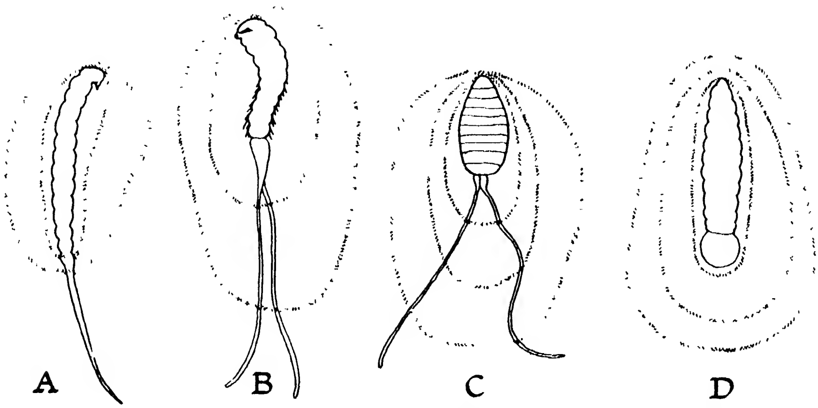

FIG. 182.—Parasitic larvae immersed in cultures of *Polytoma uvella*, showing successive positions at which bands of aggregation are formed (*after* THORPE)

A, 1st instar larva of *Omorgus mutabilis* (Hym. Ichneumonidae); *B*, 3rd instar larva of *Cryptochaetum iceryae* (Dipt. Agromyzidae); *C*, last instar larva of the same; *D*, late 2nd instar larva of *Apanteles popularis* (Hym. Braconidae).

it [58, 160]; but so far as its respiratory function is concerned, it can be said that it is of little importance. Using the aggregation the flagellate *Polytoma*, and the luminescence of *Bacillus phosphorescens*, as indicators of oxygen uptake, Thorpe [141] concluded that the 'tail' of 1st instar Ichneumonids is of no importance in respiration; the flagellates pay no attention to it at all (Fig. 182, A). The caudal vesicle of Braconids (an evagination of the wall of the hind gut which is prolapsed through the anus, and is filled with blood that is constantly taken up by the hindmost chamber of the heart (Fig. 183)) is the site of rather more active oxygen uptake than the rest of the body surface (Fig. 182, D); but even when large and supplied with a good blood circulation, as in *Apanteles* and *Microgaster*, it cannot be responsible for more than about one third of the total uptake,[141] and much of this may perhaps be associated with its own oxygen requirements.[76] The 'tails' of the Agromyzid *Cryptochaetum*, which are well supplied with fine tracheae, are also rather more important in oxygen absorption, especially towards their base, than other parts of the cuticle (Fig. 182, B, C); carbon dioxide, as in aquatic forms, being given off more or less equally over the general body surface.[141] The main function of these organs is perhaps to be sought elsewhere (p. 284).

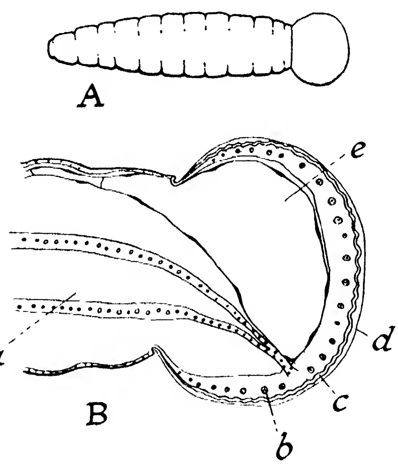

FIG. 183

A, larva of *Apanteles* showing vesicle at hind end; *B*, longitudinal section of vesicle in *Apanteles glomeratus* (*after* THORPE). *a*, mesenteron, closed behind; *b*, proctodoeum covering the vesicle; *c*, cuticle; *d*, outer membrane formed from persistent amnion; *e*, posterior chamber of heart occupying the vesicle.

Respiration of atmospheric air—We have already seen that many aquatic larvae are 'metapneustic',[112] breathing only through the hindmost pair of spiracles. This condition is paralleled among the parasitic Hymenoptera by *Blastothrix*, *Encyrtus* and other Chalcids during their 1st instar. In these the pedicel of the egg protrudes externally through the body wall of the host and functions as a kind of respiratory tube into which the larva inserts its posterior spiracles in order to breathe the atmospheric air (Fig. 184, A).[141] The respiration of many Tachinid larvae is similar; but here the respiratory tube is formed by the epidermis of the host spreading inwards over the surface of the larva and so investing it in a cuticular sheath (Fig. 184, B).[114]

Finally, just as certain aquatic insects obtain their oxygen from the intercellular gases of aquatic plants, so there are parasitic larvae which tap the tracheal system of their host. This is very common among Tachinids (*Gymnosoma*, &c.[87]). Where the trachea is ruptured by the larva, its epithelium again

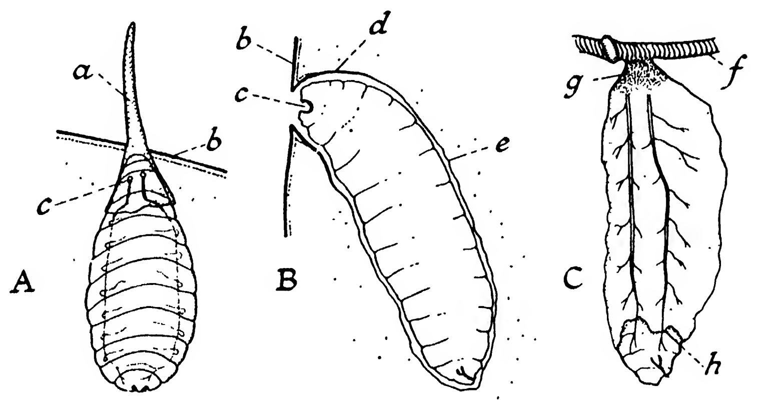

FIG. 184.—Breathing of atmospheric air by endoparasitic larvae

A, 1st instar larva of *Blastothrix* with spiracles still in chorion of egg whose pedicel protrudes through the integument of the host (*after* IMMS); B, larva of *Thrixion* enclosed in integumental sheath and breathing through respiratory funnel at the site of the entrance hole into the host; C, Tachinid larva partially enclosed in tracheal sheath and attached to tracheal trunk by means of secondary respiratory funnel (*from* IMMS *after* PANTEL). *a*, pedicel of egg; *b*, integument of host; *c*, spiracles; *d*, primary respiratory funnel; *e*, integumental sheath; *f*, trachea of host with spiracle; *g*, secondary respiratory funnel; *h*, margin of tracheal sheath.

spreads inwards to form a cuticular siphon or sheath around the parasite (Fig. 184, C).[28, 87, 114] In the last larval stage of the Chalcid *Encyrtus infelix*, the tracheae of its Coccid host become perforated by some unexplained mechanism in the neighbourhood of the anterior and posterior spiracles of the larva, and a sheath is formed which completely encloses it.[141] Whatever the mechanism of formation of this sheath, the final result is analogous to the sheaths formed by the outgrowth of epidermis or tracheal wall around Tachinids.

THE RESPIRATORY FUNCTION OF THE BLOOD

As we have already seen, the walls of the tracheae are permeable to gases,[34, 83, 165] particularly to carbon dioxide. There must therefore be some respiratory exchange between the haemolymph and the air throughout the tracheal system. And at the tracheal endings, except where these are intra-

cellular or very closely applied to the surface of the cells, the blood must always play the part of intermediary between the lumen of the trachea and the cytoplasm of the cells. How important this part shall be will depend on the anatomical arrangements. Thus in the ovary of some insects there is a considerable blood sinus between the membrane bearing the tracheal capillaries and the ovarioles ; whereas in other species the membrane lies close against the ovaries. In yet other cases, for example the pupa of *Sphinx ligustri*, there are no tracheae going to the ovary ; the blood, aerated elsewhere, plays the sole part in the supply of oxygen.[126] This must apply also, of course, to those insects in which the tracheae are wanting or filled with fluid.

The so-called 'blood gills' of aquatic and parasitic insects seem to be of small importance in respiration (p. 196) ; but the blood which circulates through them must carry away some oxygen in solution ; and the increased rate of heart beat which occurs in water poor in oxygen in the larvae of Trichoptera,[95] and in *Acentropus* (Lep.),[111] has been ascribed to this cause.

'Tracheal lungs'—In some insects the anatomy of the tracheal system suggests that it is adapted to aerate the blood ; that it serves as a lung. In the larva of *Hypoderma* (Dipt.) the tracheae end abruptly and break up into bunches of long, slender capillaries which seem to lie free in the body cavity and not to invest the cells [156] ; and the same is seen in the tracheae from the anterior spiracles of Muscid pupae.[159] Where the aorta of the honey-bee passes through the stalk of the abdomen, it is excessively convoluted and very richly supplied with tracheae.[54] In the metapneustic larvae of Diptera-Nematocera (in *Ctenophora*,[150] *Psychoda*,[38] Culicidae (Fig. 185),[69] Tipulidae,[23, 56, 163] &c.) a rich network of fine tracheae arises from the main tracheal trunks at the posterior extremity of the abdomen and invests the hindmost chamber of the heart, or forms a meshwork around the posterior opening. But in none of these cases is it known whether a significant fraction of the respiratory exchange takes place by this route.

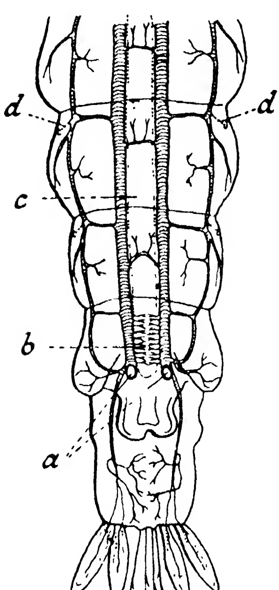

FIG. 185.—Hind end of mosquito larva, *Anopheles*, showing the rich tracheal supply to the posterior extremity of the heart (*after* IMMS)

a, spiracles ; b, tracheae to posterior chamber of heart ; c, position of heart ; d, stigmatic cords to the functionless spiracles.

In certain insects which have lost the power of flight, such as *Nepa* and *Gryllus*, the dorsal longitudinal muscles of the thorax atrophy, while their abundant tracheal supply persists. This has given rise to the impression that these vestigial muscles function as 'tracheal lungs'.[60] But they are not adapted structurally to the rapid aeration of the blood, and this does not, in fact, flow through them.[80] On the other hand, the blood sometimes circulates along the course of the tracheal system. In *Sphinx convolvuli* and *Vespa crabro* it circulates around the air sacs ; and wherever these sacs give off tracheoles, the circulating blood follows their course between the muscle bundles. It circulates also along the 'inverted tracheae' (p. 184) which traverse the rigid air chambers in the thorax of these insects.[21] And

it has been suggested that the wings of Lepidoptera serve as respiratory organs, the blood which circulates through them being aerated from the tracheal system.[119]

Haemoglobin in insect blood—It is evident from the foregoing discussion that the blood of insects is often called upon to transport oxygen ; the question arises whether it contains an oxygen carrier analogous to the haemoglobins or haemocyanins of other animals. In the larva of the honey-bee the oxygen capacity of the blood is no greater than can be accounted for by physical solution [13] ; the blood of *Dytiscus* and *Hydrophilus* normally contains only minute quantities of oxygen [11] ; but it is possible that it may be otherwise in other insects.

Appreciable amounts of copper are present in the blood and tissues,[109] but there is no evidence that this is in the form of haemocyanin. On the other hand, haemoglobin occurs free, that is, not contained in corpuscles, in the blood of certain Chironomid larvae ; and particularly in those species which live in mud at the bottom of pools, in an environment very poor in oxygen.[102] It has been suggested that this haemoglobin serves as a store for oxygen during times when dissolved oxygen is scarce [103] ; but the quantity of oxygen that could be stored in this way by a larva of *Chironomus* would last the insect only about 12 minutes.[93] At all ordinary tensions of oxygen the haemoglobin in the blood is fully saturated, and therefore plays no part as a carrier ; but at tensions of 7 mm. and under, that is, at less than 1 per cent. of an atmosphere pressure of oxygen, the haemoglobin becomes partially reduced. There is clear evidence, therefore, that this pigment does function as a carrier, enabling the blood to bind chemically sufficient oxygen for the needs of the animal when the tension of oxygen becomes so low that the necessary amount cannot be supplied by physical solution.[93]

The oxygen tension at which the oxygen consumption begins to fall off, varies greatly in different species of Chironomid larvae. If the insect is kept moist and exposed to the air, this happens in *Prodiamesa praecox* at an oxygen pressure of 6–7 per cent. of an atmosphere ; in *Chironomus plumosus* at 1–2 per cent. ; in *Eutanytarsus inermipes* at a still lower level.[61] These differences are dependent in part upon the thickness of the body wall ; but they are also related to the presence or absence of haemoglobin, the species with haemoglobin being the more resistant.[115] In these the oxygen uptake begins to fail when the haemoglobin ceases to be saturated. It is possible, though this has not been proved, that the haemoglobin of different species has a different dissociation curve, and is therefore functional over a different range of oxygen tension.[61] In *Chironomus gregarius*, haemoglobin first appears during the 2nd instar and the resistance of the larva to oxygen lack increases as the formation of haemoglobin proceeds.[115] In *Chironomus thummi*, exposure to 20 per cent. carbon monoxide (which renders the haemoglobin functionless by combining firmly with it) first affects the oxygen uptake markedly if less than 11 per cent. of oxygen is present ; whereas in larvae recovering from asphyxia, the effect is visible when 21 per cent. of oxygen is present.[61] This illustrates again that it is only under conditions of oxygen want that the haemoglobin is active as a carrier.

THE REGULATION OF RESPIRATORY MOVEMENTS

We must now consider the nature of the stimuli which control the respiratory movements of insects, and the nervous mechanisms by which they are co-ordinated. The simplest case is that of an insect which shows no pumping movements and regulates its respiration solely by opening and closing the spiracles. The control of these movements was first studied in the cockroach,[62] but the most detailed investigation has been carried out in the flea.[168] This insect is so small that diffusion takes place very rapidly, and the respiratory responses are correspondingly quick.

Chemical control of the spiracles—The spiracular movements of the normal flea have already been described (p. 190); we shall now consider the

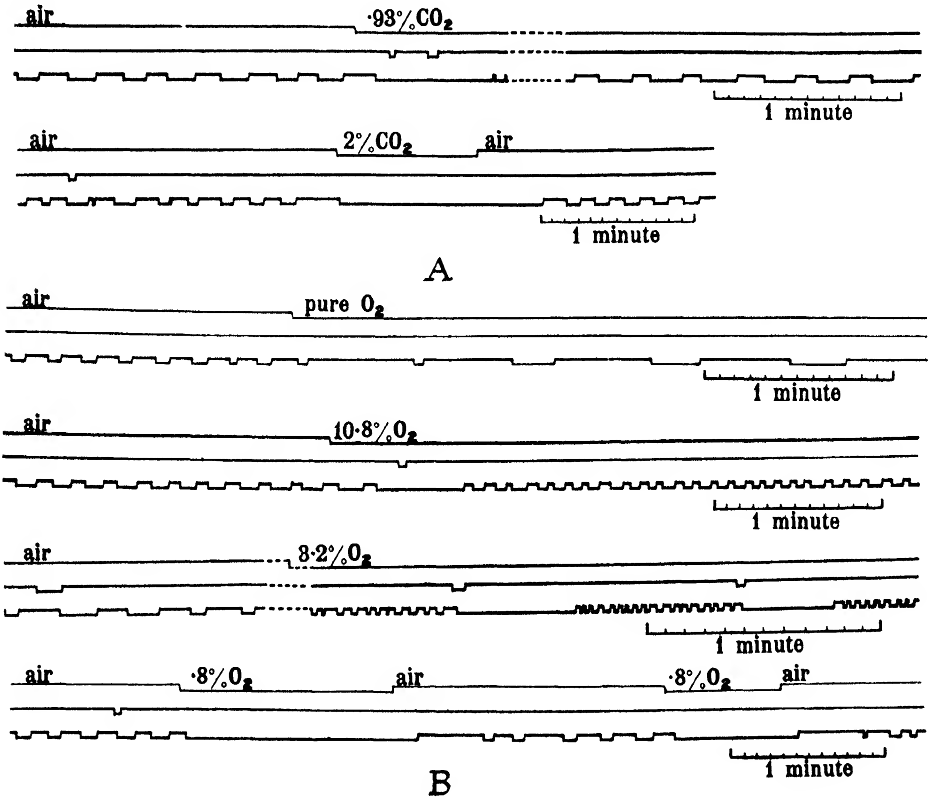

FIG. 186

A, effect of different concentrations of carbon dioxide on the opening and closing of the eighth abdominal spiracle of *Xenopsylla*; *B*, effect of different concentrations of oxygen on the same spiracle. (*After* WIGGLESWORTH.) In each tracing the upper line shows the change in gas mixture, the second line shows periods during which the flea struggled, the lower line shows opening (downwards) and closing of the spiracle. Note the prolonged opening caused by struggling in 3·2 per cent. oxygen.

regulation of their rhythmical opening and closing. Each act of opening or closing is the immediate result of chemical stimuli; there is no rhythmical discharge from the nervous system causing opening and closing at a given rate. Further, both the exhaustion of the supply of oxygen in the tracheal system during the period of closure, and the accumulation of carbon dioxide in the tissues, contribute to the regulation. In a given flea the length of time the

spiracles remain closed is more or less proportional to the oxygen content of the air breathed, and below 1 per cent. of oxygen they are open almost all the time. But the duration of the open period is determined largely by the amount of carbon dioxide that has accumulated during the period of closure. Hence the length of the open period is more or less proportional to the length of the closed period which has preceded it; in 1·8 per cent. of oxygen the spiracles close again almost instantly, in pure oxygen they remain open for perhaps 15 or 20 seconds (Fig. 186, B). This is because during the time the spiracles are closed, the carbon dioxide is present mainly in solution in the tissue fluids, and some time is necessary for it to diffuse out when the spiracles open. Thus carbon dioxide has the same effect in slowing up the responses as it has in regulating the respiration of mammals. The addition of say 1 per cent. of carbon dioxide to the outside air prolongs the open period some 50 per cent., by delaying this process of diffusion; at a concentration of 2 per cent. the spiracles are kept permanently open (Fig. 186, A).[168] For this reason the susceptibility of insects to fumigants is increased by a deficiency of oxygen or an excess of carbon dioxide.[31]

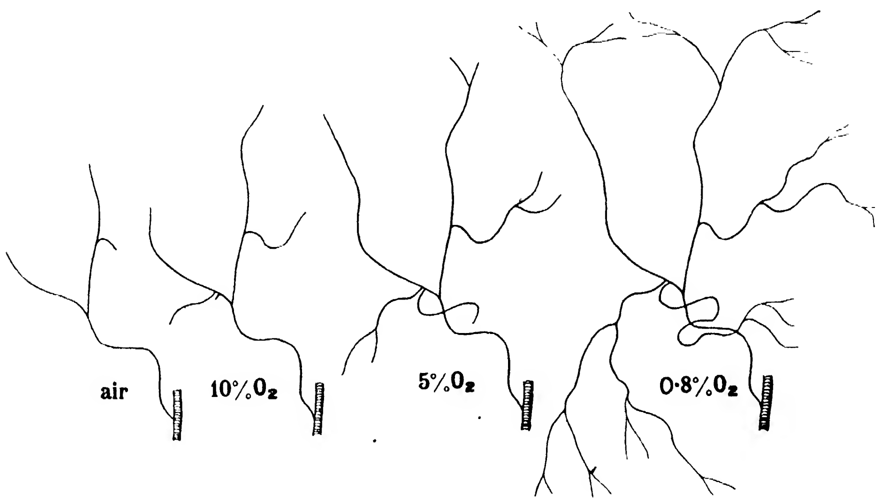

FIG. 187.—Extent of gas in one group of tracheoles in abdomen of flea at rest, exposed to different concentrations of oxygen (*after* WIGGLESWORTH)

The effective stimulus both in oxygen want and carbon dioxide excess is probably the acidity of the tissue fluids. For lactic acid is known to be produced in insects in the absence of oxygen (p. 343), and the effect of oxygen want on the spiracles can be simulated by introducting lactic acid into the blood of the insect.

This same accumulation of metabolites through incompletė oxidation causes an increase in the osmotic pressure of the blood, which in turn, in some insects, results in a removal of fluid from the tracheal endings (p. 186); and this effect, by increasing the respiratory surface, will provide a further adaptation to conditions of oxygen want (Fig. 187).

Nervous control of the spiracles—The spiracular sphincters are controlled by segmental nerves coming from the nerve cord, and the stimuli affecting their movements presumably act upon respiratory centres in the

nervous system. In the flea the main centres controlling the abdominal spiracles lie in these abdominal ganglia, but the sensitivity of these centres to carbon dioxide or to oxygen want is increased if the ganglia of the thorax are intact. The nervous system, so far as it controls respiration, is highly integrated ; this is apparent from the fact that with weak stimuli the opening is confined to particular spiracles (p. 190). But slow reactions of the spiracles to nitrogen or to carbon dioxide may persist after they are completely severed from the central nervous system,[62, 168] and this suggests that there must be some peripheral nervous mechanism, perhaps a nerve ganglion associated with each spiracle (as has been described in the lepidopterous larva *Cossus* [88]) which is normally under the control of centres in the nerve cord.

The prolonged opening of the spiracles which follows muscular exertion in the flea, seems always to be the result of chemical stimulation, probably again the combined effect of carbon dioxide production and oxygen want, and is never due to a nervous stimulus coincident with the onset of muscular action. The rapid rhythm of spiracular movements which occurs when the temperature is raised can be explained by the combined effect of temperature on the intensity of metabolism and on the rate of diffusion.

Chemical control of pumping movements—When we turn to insects in which pumping movements are added to regulation by diffusion, the phenomena

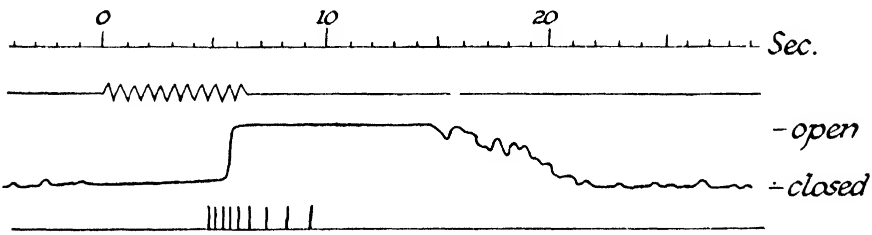

FIG. 188.—Effect of struggling on respiratory movements in *Periplaneta* (*after* HAZELHOFF)

Upper line : time in seconds. Second line : struggling movements. Third line : degree of opening of first thoracic spiracle. Lower line : ventilating movements.

become more complicated (Fig. 188). In the cockroach [62] at low concentrations of carbon dioxide, regulation of the spiracles resembles that in the flea : 2 per cent. CO_2 in air causes all the spiracles to remain permanently open. But on raising the CO_2 to 10 per cent., slow pumping movements of 8–10 per minute begin, even in the insect at rest ; and then the spiracles show rhythmic movements—the second thoracic and the last seven abdominal spiracles closing for a period at the beginning of expiration, the first thoracic and first abdominal remaining open all the time. At 15 per cent. CO_2, the pumping movements increase to 90–120 per minute and the spiracle movements continue. At 20–30 per cent. CO_2, respiration is at the rate of 150–180 per minute and the spiracles no longer close. Besides varying in rate, the respirations vary also in depth of movement.[50]

These results show how complex is the regulation of respiration in such an insect. At concentrations of carbon dioxide great enough to cause pumping movements, the spiracles no longer remain permanently open, but a rhythmic closure of certain of them supervenes ; a process which, as we have seen (p. 194), drives a stream of air through the tracheal system. There is a true

nervous co-ordination between ventilating and spiracular movements; for both persist unchanged in insects with all the abdominal contents removed with the exception of the ganglia and muscles.[50]

In the control of the pumping movements in the cockroach, as in the control of the spiracles, both oxygen want and carbon dioxide excess can act as stimulus. At 5 per cent. of oxygen in nitrogen, pumping movements and the spiracular rhythm begin; and at a given partial pressure of carbon dioxide, dyspnoea is greater, the less the partial pressure of oxygen: in 15 per cent. CO_2 in *air* dyspnoea begins in 8 seconds and shows a maximum frequency of 120 per minute; whereas in 15 per cent. CO_2 in *oxygen* dyspnoea begins in 60 seconds and reaches a frequency of only 45 per minute.[62]

Stimulation of respiratory movements by both high carbon dioxide and low oxygen concentrations, has also been observed in other insects; grasshoppers show a violent hyperpnoea in oxygen containing 10 per cent. of carbon dioxide [84]; *Dixippus morosus* shows respiratory movements when the oxygen content of the air falls below 3–4 per cent., besides responding to high carbon dioxide concentrations [25, 135]; the ventilation of the gill chamber of *Aeschna* larvae is brought about by water poor in oxygen or water containing minute quantities of free carbonic acid, changing the *p*H from 7 to 6·75.[135]

Control of respiratory activities of aquatic insects.—On the other hand, the rhythmical vibrations of the gill lamellae of Ephemerid nymphs,[9] the body movements of the aquatic larva of *Nymphula* (Lepidoptera),[161] and the movements by which *Phryganea* larvae (Trichoptera) ventilate their tubes,[33] seem to be induced solely by the low oxygen content of the water. When the aquatic bug *Corixa* is submerged, it will often direct a current of water over its ventral air store (p. 201) by movements of its legs. These movements are said to be due always to the accumulation of carbon dioxide and not to lack of oxygen. Whereas the stimulus which causes this insect to rise to the surface to replenish its supply of air, is said to be the diminishing size of the air bubble it carries.[18] In *Notonecta*, likewise, it is the diminution of buoyancy due to the contraction of the air store which is the stimulus driving it to the surface; an artificial increase in air pressure above the water causes *Notonecta* to rise immediately.[17] On the other hand, *Naucoris* is said to be made to rise by lack of oxygen, and if kept in water exposed to pure oxygen it will die, because this stimulus never occurs until all its air store has been consumed and water has entered the tracheal system.[175] It is lack of oxygen, also, and not carbon dioxide excess, which causes the surface breathing ('Notatmung') of *Aeschna* larvae, which come to the surface to breathe if the oxygen content of the tracheae falls to 3·2 per cent.,[155] and the ascent of the mosquito larva [75] and the larva,[86] and adult [63] of *Dytiscus* to the water surface.

Nervous control of pumping movements—These chemical stimuli exert their action upon respiratory centres in the nervous system, the distribution of which recalls that of the centres controlling the spiracles in the flea. It was shown by Marshall Hall (1842), and has been repeatedly confirmed since,[166] that the isolated abdominal segments of *Libellula* and other insects can still perform respiratory movements; and the isolated ganglia of *Dytiscus* and *Aeschna* show rhythmical changes in electrical potential which doubtless indicate the activity of respiratory centres.[1] The abdominal centres are sometimes termed 'primary centres'; they are always rather insensitive to stimuli.

In the larva of *Aeschna* the isolated abdomen is quite indifferent to changes in the oxygen tension of the medium [135, 155]; in *Dixippus* the abdominal centres will respond to oxygen want with pumping movements, but they are far less sensitive than the intact insect. The ' secondary centres ', which are responsible for the more sensitive reactions to chemical stimuli, are situated in the thorax, usually in the prothoracic ganglion (in *Aeschna* [155] and in *Dixippus* [135]). Decapitation, involving removal of the brain and suboesophageal ganglion, causes only a temporary and variable disturbance of the respiratory movements.[166]

Probably there is only a quantitative difference between the ' primary ' and ' secondary ' respiratory centres. If the thorax of the locust *Schistocerca* is separated from the abdomen, the respiratory rhythm of the thorax is unchanged, while that of the abdomen is slowed. The ' secondary ' centres in the thorax are really the ' primary ' centres for the thoracic respiratory movements; and being more sensitive to chemical stimuli they set the general pace of the respiratory rhythm.[50] On the other hand, the movements of the gills of *Cloëon* nymphs are said to be controlled by a series of stimulatory and inhibitory centres with definite localization.[3]

But besides being influenced by chemical stimuli the rhythmic discharges of the respiratory centres are affected by nervous stimuli from elsewhere in the system. Acceleration of respiration may be brought about by any outside stimulus (mechanical, sudden illumination, &c.) to which the insect may be exposed [10, 50, 135]; and respiration may be accelerated, or the movements increased in amplitude, before or at the very outset of muscular effort. Thus an analysis of the respiratory movements during and after flight in a great number of insects, has shown that both reflex nervous stimuli from other motor centres, and chemical stimuli resulting from increased metabolism, contribute to the augmented ventilation. [50]

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Chapter X

The Circulatory System and Associated Tissues

THE BODY cavity of insects is of twofold origin ; being formed in the embryo by the fusion of the lumen of the coelom sacs with the epineural sinus or haemocoele. It lacks the epithelial lining of a true coelom, and it contains circulating blood. The organs and tissues are thus exposed freely to a stream of fluid which percolates among them. Most of the organs in question, the epidermis and tracheal system, the alimentary canal and Malpighian tubes, the nervous system, muscles, glands and gonads, are dealt with elsewhere ; but there are a number of tissues, the haemocytes or blood cells, the pericardial cells and other ' nephrocytes ', the oenocytes, fat body and mycetocytes, which are in relation only with the blood. These must perform their functions solely through exchanges with the circulating haemolymph ; and although they doubtless play widely different parts in metabolism, these tissues may be conveniently considered here.

CIRCULATORY SYSTEM

The Dorsal Vessel—The circulation of the blood is maintained by a system of muscular pumps and fibro-muscular septa (Fig. 189). The most important and in many insects the only organ responsible for its transport is the dorsal vessel, which collects blood from the abdominal cavity and discharges it in the head. The dorsal vessel is divided rather indefinitely into a posterior region, the heart, which has a pair of valved openings or ostia in each body segment, and an anterior region, the aorta, a closed tube. In some insects, such as the cockroach, the heart may extend throughout the abdomen into the hind part of the thorax, and possess as many as 13 pairs of ostia ; in other insects, such as the nymphs of Agrionid dragon-flies, it is reduced to a single chamber with a single pair of ostia ; and between these extremes all intermediate stages exist (in *Aeschna* nymphs, a single chamber with 2 pairs of ostia [190] ; in different species of Ephemeroptera 1–4 pairs of functional ostia [113] ; in the honey-bee 5 pairs [45] ; in the larva of *Corethra* 7 pairs,[100] &c.) (Fig. 190).

In its simplest form, as seen in the larvae of Diptera, the wall of the dorsal vessel is made up of a single layer of cells, their substance differentiated into circular fibrillae, indistinctly striated, enclosed between two homogeneous membranes probably derived from the sarcolemma.[123] Or the muscles may consist of interlacing striated fibrillae embedded in sarcoplasm, as in *Aeschna* [190] ; or longitudinal and circular fibrillae, as in *Nepa* [57] ; and outside the outer membrane there is generally an adventitia of connective tissue carrying the tracheoles (Fig. 191).[147] The heart and aorta commonly have the same histological structure ; both are contractile.

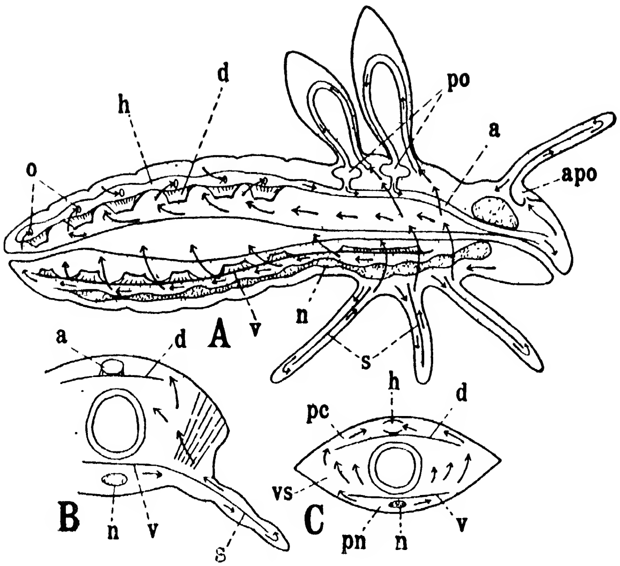


Fig. 189

A, insect with fully developed circulatory system, schematic; *B*, transverse section of thorax of the same; *C*, transverse section of abdomen. Arrows indicate course of circulation (*based largely on* Brocher). *a*, aorta; *apo*, accessory pulsatile organ of antenna; *d*, dorsal diaphragm with aliform muscles; *h*, heart; *n*, nerve cord, *o*, ostia; *pc*, pericardial sinus; *pn*, perineural sinus; *po*, meso- and metathoracic pulsatile organs; *s*, septa dividing appendages; *v*, ventral diaphragm; *vs*, visceral sinus

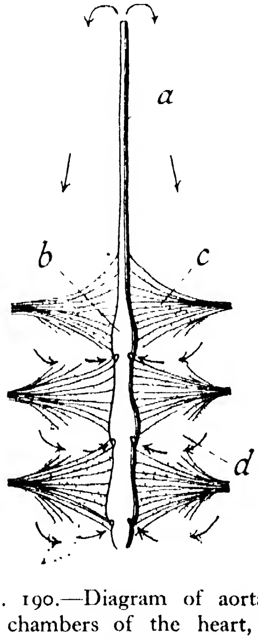


Fig. 190.—Diagram of aorta with three chambers of the heart, dorsal view (*after* Snodgrass)

a, aorta; *b*, heart; *c*, alary muscles; *d*, dorsal diaphragm.

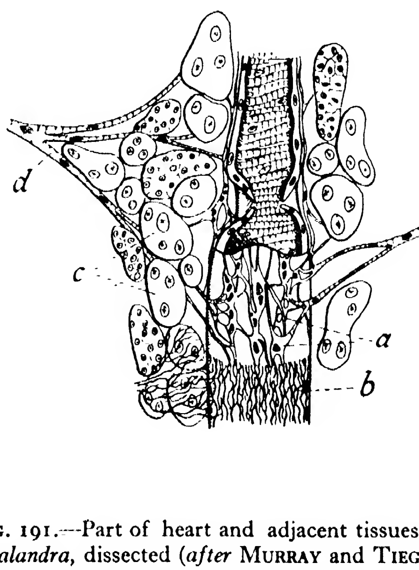


Fig. 191.—Part of heart and adjacent tissues in *Calandra*, dissected (*after* Murray and Tiegs)

Cavity of heart exposed above to show contractile tissue and a pair of valves guarding two ostia. *a*, adventitia (pericardium); *b*, elastic tissue (removed elsewhere); *c*, pericardial cells; *d*, alary muscles.

The heart is tied to adjacent structures by radiating filaments. It may be bound directly to the dorsal body wall, as in *Aleurodes* (Fig. 192, A),[170] or attached to it by threads, as in *Lepisma*,[18] *Corethra*,[100] *Nepa* (Fig. 192, C),[57] and many other insects. It may be connected to the lateral body wall in each segment by fan-shaped strings, as in *Corethra*,[100] or by the pericardial septum (p. 222), when this is inserted directly into the sides of the heart, as in *Nepa*,[57] *Aleurodes*,[170] &c. More often this membrane lies below the heart, which is then attached to it by elastic fibres running downwards and outwards, as in Ephemeroptera,[134] *Dytiscus* (Fig. 192, D),[15] &c.

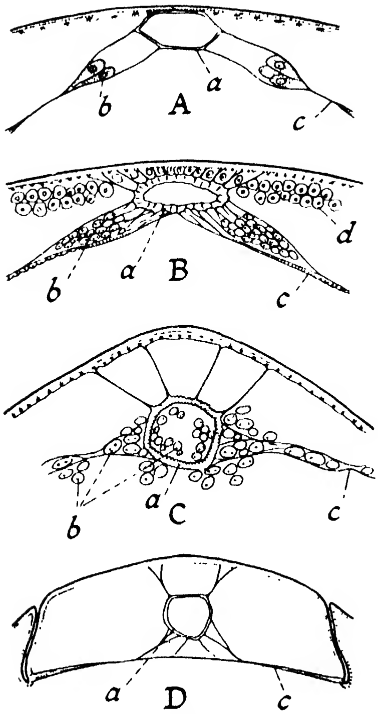


FIG. 192.—Examples of different methods of support for the heart, showing the effect of the pericardial septum in causing dilatation

A, *Aleurodes* (*after* WEBER); *B*, *Apis* (*after* SNODGRASS); *C*, *Nepa* (*after* HAMILTON); *D*, *Dytiscus* (*from* WEBER *after* OBERLE). *a*, heart; *b*, pericardial cells; *c*, pericardial septum; *d*, fat body cells.

Ostial valves—The ostia are slit-like openings in the sides of the heart, with their margins prolonged inwards to form valves. These valves serve primarily to prevent the reflux of blood into the body cavity when the heart contracts. Their action is well seen in the larva of *Corethra*.[162] Here each ostial lip has a unicellular thickening which runs into a thread attached to the inner wall of the heart. When the heart dilates the valves are widely separated and the blood enters (Fig. 193, A); when dilatation is complete (diastole) the valves are closed and stand out at right angles to the wall (Fig. 193, B); during contraction or systole they become evaginated as far as their attached threads will permit, and are forced together so that no blood can escape (Fig. 193, C).

Heart chambers—In most insects the heart is a continuous tube (Fig. 194, A); rarely is it divided into chambers. Chambers are sometimes formed by the prolongation inwards of the ostial valves. In nymphs of *Cloëon* the anterior lips of these valves are so elongated that they meet across the lumen, and serve to some extent to prevent reflux of blood within the heart (Fig. 194, B); but they are not entirely competent, for some blood passes back again during diastole.[53, 134] Occasionally, valves may be developed from folds of the inner wall of the heart, independent of the ostia. In *Chironomus dorsalis* a pair of such valves separates the heart from the aorta (Fig. 194, C)[134]; in Tipulids there is a pair in

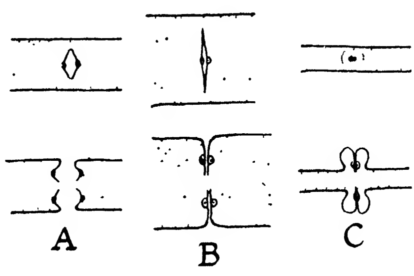


FIG. 193.—Ostial valves in larva of *Corethra* at different phases of the heart beat

Above, lateral view; below, dorsal view. *A*, beginning of diastole; *B*, complete diastole; *C*, complete systole (*after* TZONIS).

each segment just anterior to the ostia (Fig. 194, E).[172] In some species of *Chironomus* and *Tanypus*[134] a pair of muscular pads project inwards between successive ostia; the heart contracts most strongly at this level, causing an apparent division into chambers (Fig. 194, D). Another type of intra-cardiac valve is seen in the larvae of *Sarcophaga carnaria*, the Tachinid *Compsilura*, &c., and consists of two cushions of large vacuolated cells projecting far into the lumen (Fig. 194, F).[124]

Extremities of the dorsal vessel—In most insects the heart is closed behind; but posterior ostia occur in *Aleurodes*,[170] *Corethra*, *Culex*, *Ctenophora*, &c., and a median posterior opening with well developed valves is present in the Tachinid *Ceromasia* (Fig. 194, F),[124] and in the larva of *Tipula maxima*.[16]

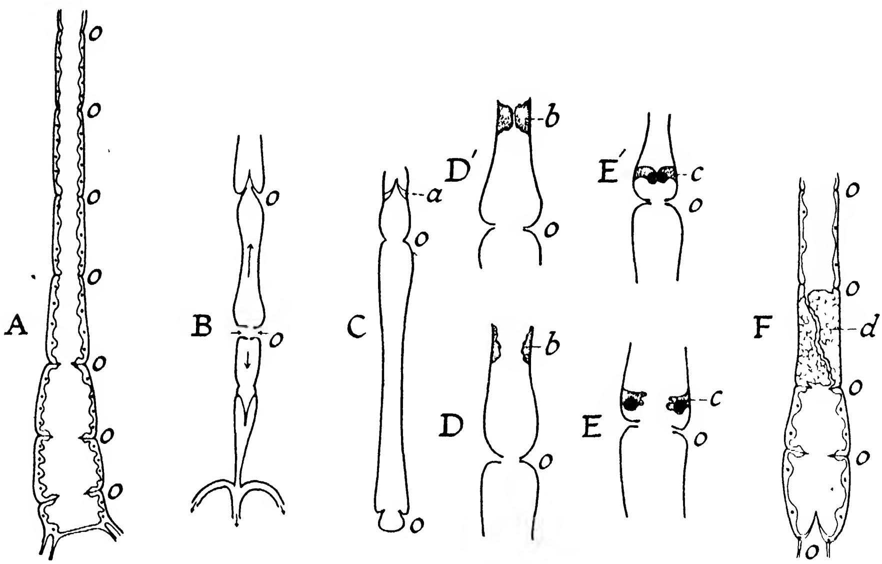


FIG. 194.—Examples of valves in the heart

A, larva of *Thrixion* (Tachinidae); a heart with no valves between chambers (*after* PANTEL). *B*, *Cloëon dipterum*; the ostial valves modified to provide interventricular valves; in the hindmost chamber they are directed backwards, the blood flowing into the three vessels to the caudal filaments. *C*, Chironomid larva with separate interventricular valves (*a*) between heart and aorta. *D*, Chironomid larva with muscular pads (*b*) which separate the chambers during systole (*D′*) (*after* POPOVICI-BAZNOSANU). *E*, larva of *Tipula* with separate valve flaps (*c*) in front of ostia. *E′*, the same in systole (*after* WETTINGER). *F*, larva of *Ceromasia* (Tachinidae) showing 'preventricular cushion' (*d*) and terminal posterior ostium (*after* PANTEL). *o*, ostia.

In Ephemerid nymphs the blood flows backwards in the hindmost chamber, through a pair of backwardly directed valves, into the vessels to the caudal filaments (Fig. 194, B).[134]

In front, the aorta ends by discharging into the body cavity, often by way of a sinus which accompanies the oesophagus beneath the brain; in the Reduviid *Rhodnius*, it discharges downwards just behind the brain into a sinus which runs forwards through the oesophageal ring and backwards around the corpus allatum (Fig. 195).[178] In the cockroach,[126] Phasmids,[147] &c., the aorta continues as an inverted gutter to fuse with a transverse band of muscle in the head and discharges ventrally in front of the brain (Fig. 197). In the honey-bee, some blood escapes from the aorta before it enters the oesophageal ring, and bathes the back of the brain.[45]

The pericardial septum—The direction of the blood stream outside the dorsal vessel is controlled in part by fibro-muscular septa. A dorsal diaphragm or pericardial septum lies immediately below the heart and is attached laterally to the terga. It often contains muscle fibres; typically these form the alary muscles arranged in a fan-shaped manner in each segment, widely spread beneath the heart, converging towards the body wall. The ostia of the heart generally open into that part of the body cavity, the pericardial sinus, which is cut off by this septum; and in order to reach this space the blood must either pass through openings in the membrane, which is usually fenestrated, as in the cockroach,[15] or, if the membrane is imperforate, as in grasshoppers,[106] it must flow round the posterior border. In some Orthoptera, *Pachytylus*, *Locusta*, in addition to the ordinary lateral ostia of the heart, there are five pairs which open ventrally and collect blood directly from the perivisceral cavity.[87] In

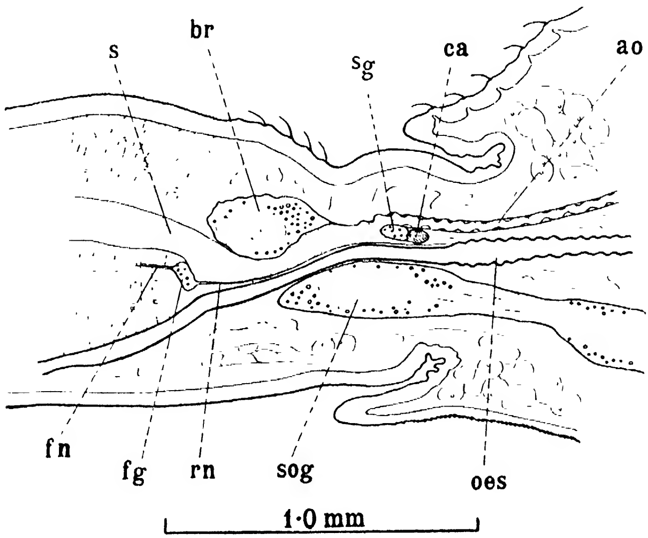


FIG. 195.—Longitudinal section of part of head and thorax of *Rhodnius* showing the ending of the aorta (*after* WIGGLESWORTH)

ao, aorta; *br*, brain; *ca*, corpus allatum; *fg*, frontal ganglion; *fn*, frontal nerve; *oes*, oesophagus; *s*, blood sinus into which aorta discharges; *sg*, sympathetic ganglion; *sog*, suboesophageal ganglion.

some insects (many Dipterous larvae, Hemiptera, Anoplura, &c.) the pericardial septum is attached directly to the heart; sometimes it is reduced to a few fibrous strands, as in *Corethra* larvae.[100]

Circulatory mechanism—The blood is aspirated through the ostia by the dilatation of the heart. In the honey-bee, and doubtless other insects, this aspirating movement is greatest in the wide posterior segment with muscle-thickened walls [45] and here it sets up transverse currents towards the ostia. Dilatation is brought about by the elasticity of the fibres radiating from the heart. The pericardial septum is convex upwards; hence when the alary muscles contract they will enlarge the pericardial sinus,[53] displace the blood towards the heart, and by drawing upon the ventral wall of the heart, aid its dilatation.[123, 147] Or, if they merely maintain a constant tension in the septum, they will provide an elastic pull upon the fibres to the heart, and will have the same effect (Ephemeroptera,[134] *Dytiscus*,[15] *Apis* [45]).

When the heart is filled with blood, a steady wave of contraction passes from behind forwards; there is no alternation of diastole and systole in successive chambers. The blood is carried to the head and there discharged. This causes a relative increase in pressure at the anterior end of the insect; so that at the same time as blood passes forwards in the heart, it is moved backwards towards the abdomen in the general body cavity. The pressure gradients which maintain this circulation are produced by the muscular work of the heart; such gradients can exist whether the average pressure in the body cavity is greater or less than the atmospheric pressure. The circulation can be maintained in the narcotized insect, in which the general pressure is below that of the atmosphere (in *Dytiscus* [15] and in the honey-bee *Apis* [45]); but this is no reason for regarding the heart primarily as a suction pump.[113]

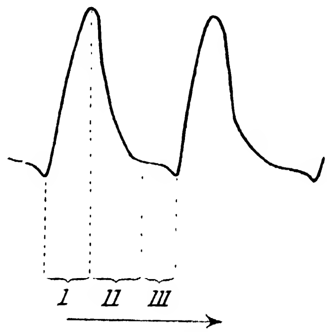


FIG. 196.—Diagram showing type of mechanical record obtainable from the isolated heart of *Periplaneta americana* (*after* YEAGER)

i, phase of systole; *ii*, phase of diastole; *iii*, phase of diastasis, with 'presystolic notch' before the ensuing systole.

Three phases in the cycle of the heart's activity at a given point in its course are described: (i) contraction or systole, (ii) relaxation or diastole and (iii) rest in the relaxed condition or diastasis.[185] The diastolic relaxation of the heart is attributed to the elasticity of its walls. But at the end of the diastolic rest, it may show a sudden further dilatation before the ensuing systole (the 'presystolic notch'). Perhaps this is caused by distension with blood driven forwards from behind, perhaps by a contraction of the alary muscles preceding the contraction of the heart wall (Fig. 196).[91, 185]

The ventral diaphragm—In Orthoptera, Odonata, Ephemeroptera, Hymenoptera, and particularly in Lepidoptera, there is another well developed fibro-muscular septum stretched across the abdomen just above the nerve cord, thus enclosing below it a perineural sinus. This membrane is capable of undulatory movements, which direct the flow of blood beneath it backwards and laterally,[15, 45] and ensure the irrigation of the nerve cord. In Libellulids this membrane is convex downwards; when it contracts the perineural sinus is enlarged [53]; in nymphs of *Cloëon* (Ephem.) it is convex upwards; its contraction will drive blood out of the ventral sinus.[134] Like the dorsal diaphragm, this membrane is often incomplete (as in the cockroach [15]) so that blood can pass through it into the perivisceral cavity; in many insects it is absent altogether.

The effect of respiratory movements on the circulation—The changes in blood pressure brought about by the respiratory pumping movements (p. 192) can seldom be equal throughout the body; they must, therefore, influence the circulation of the blood. In *Aeschna* nymphs there are two vertical transverse diaphragms in the thorax, provided with sphincters through which the oesophagus passes. At each inspiratory movement of the abdomen the blood flows backwards; during expiration it is prevented from returning by contraction of these sphincters; so that respiration actively assists the circulation. In this insect, and in Agrionid nymphs, the pulses of blood in the femur

coincide with the respiratory movements in the rectum.[15] In Ephemerid nymphs the pumping of water in and out of the rectum causes a ' ackward and forward displacement of the blood in the abdomen, but does not bring about any transport. In the legs, on the other hand, these rhythmic changes in pressure do seem to assist the circulation.[113]

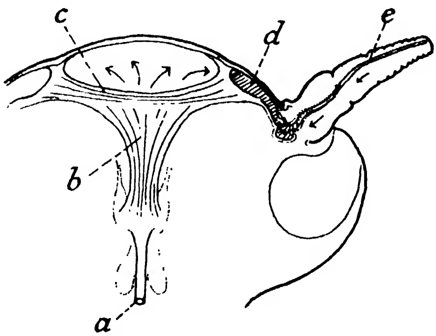


FIG. 197.—Circulation of blood in head of *Blatta* (*after* PAWLOWA)

a, aorta; b, longitudinal muscle in form of an inverted trough; c, transverse muscle; d, ampulla; e, blood vessel running from ampulla along antenna.

Circulation in appendages; accessory hearts—Special arrangements exist for irrigating the appendages. Rarely the blood is conveyed by closed vessels from the aorta: in May-fly nymphs, the blood entering the hindmost chamber of the heart divides into a forward and a backward current, and the hind end of the heart splits into three arteries which carry blood along the caudal filaments.[134] In *Vespa*, the aorta ends in the head in a transverse vessel which gives off branches to the eyes.[15]

But as a rule the appendages are supplied with blood from the sinus cavities, and it is driven through them by independent pumps. In *Periplaneta*, *Locusta*, *Stenobothrus*, &c. there are two little ampullae beneath the clypeus, each giving off a vessel which runs to the tip of the antenna (Fig. 197). Each ampulla has a valvular opening communicating with the blood space in front of the brain into which the aorta discharges. A transverse muscle with striated fibres connects the inner walls of these two sacs and acts as a dilator; the contraction of the sacs, which drives the blood up the antenna, is due perhaps to their own elasticity, perhaps to intrinsic muscles in their walls. All along these antennal vessels are small perforations through which the blood escapes.[15, 126] Similar vessels and ampullae supply the antennae of the honey-bee[45] and doubtless many other insects.

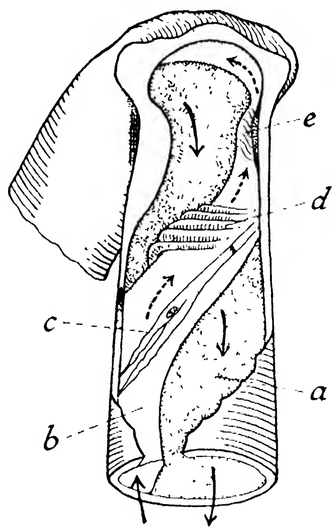


FIG. 198.—Pulsatile organ in fore leg of *Notonecta* (*after* DEBAISIEUX, simplified)

a, ventral blood sinus; b, dorsal blood sinus; c, scoloparium; d, compressor muscle; e, valvular membrane. Arrows indicate direction of blood flow.

Pulsating organs are particularly numerous in Hemiptera: they were discovered by Behn in 1834 in the legs of *Notonecta*, and have been seen in many other bugs[32]; they occur in the wings of *Nepa*[57]; there are four in each wing in *Musca*, lying on the course of the efferent veins[158]; and no doubt many more remain to be discovered. Their mode of action is not well understood, but Fig. 198 represents the probable arrangement in the limbs of *Notonecta*. A dorsal vessel running up the leg crosses the tibia obliquely just below the knee, and at this point a muscle curves round its upper border.

This muscle contracts spasmodically, driving the blood along the vessel, past a valvular membrane, to the body cavity, and aspirating blood from this cavity

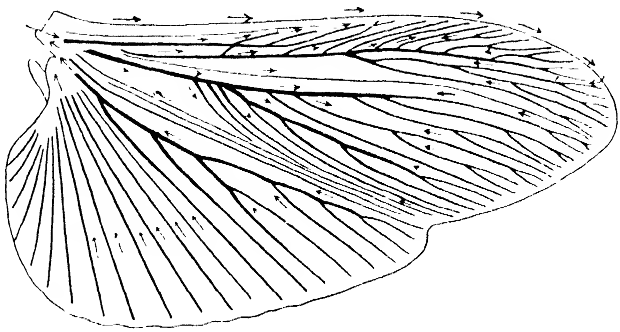


FIG. 199.—Hind wing of *Periplaneta americana* with the course of circulation of the blood indicated by arrows (*after* YEAGER and HENDRICKSON)

into the ventral sinus of the limb.[32] In the legs and gills of Ephemerid nymphs the movements of the appendages themselves seem to aid the flow of blood.[113]

Circulation in the wings of insects was observed by Henry Baker in the grasshopper as early as 1744,[182] and is probably universal. The blood flows between the tracheae and the walls of the 'veins'; it enters the wing by the costa as a rule, and returns to the body by the posterior margin, following a fairly constant path along the larger channels in the wing (Fig. 199). As we have seen, this circulation may be furthered by pumps in the wing itself, but it is effected chiefly by pulsating organs in the thorax. Such organs are present in most insects. They have been described, for example, in the meso- and metathorax of Odonata nymphs,[15] *Dytiscus* [15] and other beetles,[15] in the mesothorax of Sphingidae,[15] *Vespa*,[15] *Apis*,[45] *Tabanus*,[15] *Musca*.[158] They seem to consist of a muscular plate, which encloses a blood space beneath the dorsal wall of the thorax, often in the scutellum (Fig. 200). This space communicates with the veins of the posterior wing margin, and when the muscles relax, it dilates and blood is aspirated through the venous network of the wings; blood enters the wings, by the anterior veins, from the lateral intermuscular spaces of the thorax. When the organ contracts, the blood is expelled through a valved opening below; this may lead directly or by a tubular vessel into the aorta (in Odonata,[15] *Dytiscus*,[15] *Coccinella*,[15] the

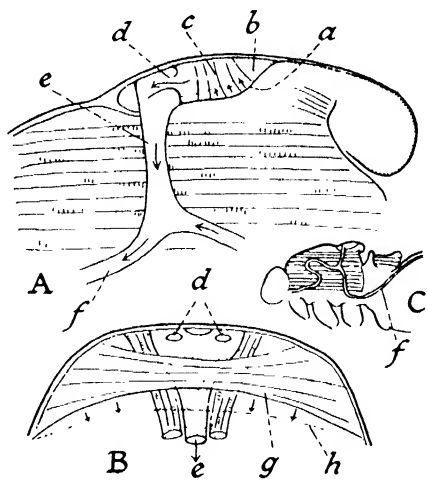


FIG. 200.—Mesothoracic pulsatile organ in *Sphinx convolvuli* (*after* BROCHER)

A, side view; B, posterior view; C, course of aorta through the thorax as seen in side view. a, pulsatile membrane contracted; b, dotted line showing position of relaxed membrane; c, elastic fibres connecting membrane to dorsal wall; d, valved orifice; e, connecting vessel running to aorta; f, aorta; g, pulsatile membrane relaxed; h, dotted line showing position of the membrane when contracted.

mesotergal organ of Sphingidae [15]), or it may simply discharge into the body cavity (in *Musca*,[158] *Tabanus*, and the metatergal organ of Sphingidae [15]).

From time to time the direction of flow in the wings may be reversed (in *Coccinella*,[15] in *Periplaneta* [182]) quite independently of the reversal in the dorsal vessel (p. 227). The mechanism of this change is not known. In May-fly nymphs the wings are irrigated by an evagination of the dorsal wall of the aorta which is itself closed and takes up no blood ; but it is surrounded by a blood space communicating with the wing veins, and when it contracts blood is sucked from the wings and pressed forward towards the head.[113]

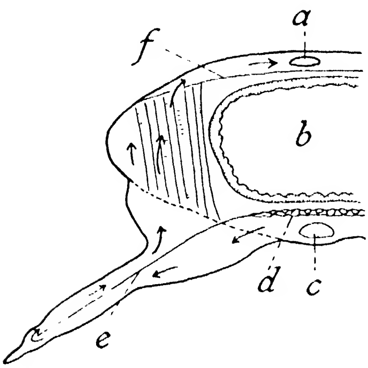


FIG. 201.—Diagrammatic cross-section through thorax of *Blatta*, showing the course of circulation through the leg (*after* BROCHER)

a, heart ; *b*, gut ; *c*, ventral nerve cord ; *d*, ventral diaphragm roofing over the perineural sinus ; *e*, septum dividing the leg ; *f*, dorsal diaphragm forming floor of pericardial sinus.

The pulsating organs of the thorax may have far reaching effects upon the movements of the blood ; in Sphingidae they are said to be more important than the heart itself.[15] In most insects the legs are divided by a membrane into two compartments, and in many forms in which pulsating organs have not been demonstrated in the legs, the blood flows down the posterior and up the anterior compartment. This movement is probably due to the fact that the posterior compartment is in connection with the perineural sinus in which the pressure is relatively high, while the anterior compartment communicates with the lateral intermuscular spaces of the thorax, in which the aspiration of the pulsating tergal organs maintains a lower pressure (in Sphingidae,[15] and in the honey-bee [45]). In the cockroach perhaps the difference in pressure between the perineural sinus and the perivisceral sinus is sufficient to maintain this flow (Fig. 201).[15]

The pulse rate in the dorsal vessel varies from many causes. In the larvae of *Sphinx ligustri* it falls during the course of development from a rate of 82 per minute in the 1st instar, 89 in the 2nd, 63 in the 3rd, 45 in the 4th, to 39 per minute shortly before maturity. During the resting period before each moult it will fall to 30. In the pupa it falls from 20 to 10 per minute, and during the winter rest ceases almost completely.[119] Similar results have been obtained with *Bombyx mori*.[107] The rate of beat is increased as the temperature is raised.[27, 46, 153] It is increased when the insect becomes active : in the imago of *Sphinx* the rate is 40–50 per minute during rest, 110–140 during activity.[119] And it is increased if the metabolic rate is high from any other cause : the respiratory metabolism of *Baetis rhodani* (Ephem.) in rapid streams is three or four times that of *Cloëon dipterum* in ponds, and the ratio of their pulse rate is 3 : 1.[44]

The accessory pulsating organs, though their movements are quite independent of the heart,[32] respond in the same way. The leg pumps in *Notonecta* are accelerated by high temperature [28] ; the mesotergal organ in *Macroglossa* beats 10–12 per minute with frequent pauses during rest, 75 per minute when the insect is disturbed, and at a rate too fast to count when it is about to fly.[15]

The rate of propagation of the beat also varies very much. In the mature larva of *Lucanus cervus* at 18° C. the pulse rate is 12–20 per minute (average 14) and the rate of propagation 19·5–44·5 mm. per second (average 27·2).[98] In the larva of *Corethra* at 17–18° C., with a pulse rate of 15–16 per minute, the waves do not move at more than 1 mm. per second, so that three systolic waves may be passing along the dorsal vessel at the same time,[162] and simultaneous pulsations take place in the backward flow of blood in the body cavity.

Automatism of the heart—The property of rhythmical contraction certainly resides in the heart muscle : there are no nerve cells or ganglia associated with the heart in *Aeschna*,[190] and *Anax* [103] nymphs or in the adult of *Belostoma* (Hem.) [103]; and yet the isolated heart, or even fragments of the heart (*Dytiscus*), continue to beat rhythmically.[39, 106] And the propagation of the contraction wave seems also to be effected wholly by the muscles ; waves are still conducted after section of the heart unless it be cut through entirely.[37]

It has been claimed that the elastic tension of the alary muscles, or even the pull of their contractions, is necessary to ensure rhythmical pulsations (in *Chironomus* larvae and Agrionid nymphs).[37] This stretching of the wall certainly modifies the contraction ; for the heart of *Dytiscus*, which beats at 30–70 per minute when intact, is slowed to 15 per minute when all the alary muscles have been cut [39]; but it is not arrested [53, 134]; and in *Apis*, the heart beat continues after section of the entire pericardial septum.[134] In nymphs of *Anax* it is said that the suspensory ligaments running to the dorsal cuticle must be intact if the heart muscle is to function.[103]

Reversal of the heart beat—All segments of the heart may show automatism.[37] But normally it is best developed at the hind end. This sets the pace ; and waves pass forwards from the hindmost chamber. It is, however, not uncommon for the beat to be reversed. This was observed by Malpighi in the silkworm, in which it begins about 48 hours before pupation, and continues at intervals until the death of the adult.[48] Reversal of this kind has been described in the pupae and imagines of many Diptera, Coleoptera, Lepidoptera and Hymenoptera.[48] It is an interesting phenomenon, firstly because it emphasizes the unity of the insect heart, the point already stressed that the heart acts as a single tube and not as a series of chambers,[48] and secondly because of the light it throws on the cause of the normal dominance of the hind end.

It has been suggested that the chief cause of reversal may be mechanical : the blockage of the ostia by the disintegrating fat body, in *Galerucella* [136]; or the obstruction of flow by the histolyzing tissues in the body cavity increasing the tension in the head and starting an antiperistalsis, in *Saturnia*.[160] But the heart upon the excised dorsum of the pupa continues to exhibit a periodic reversal.[48] It seems more likely that there is a real disturbance of automatism.

In the silkworm embryo the heart beat begins 2 days before the entrance of air in the tracheae ; but it is slow and shows no definite direction. When air appears, the beat becomes regular, and its forward direction becomes constant.[186] In the larva of the silkworm, in which reversal does not normally occur, it can be evoked by warming the anterior region of the heart, or by occluding the last two pairs of spiracles.[186] Thus it may be that the normal dominance of the posterior extremity in the larva is due to its rich tracheal supply ; in the pupa the respiratory supply to the vessel is very feeble behind; in the imago it is dense in both regions.[186] The posterior extremity of the heart in Dipterous

larvae is invested by a rich basketwork of tracheae (p. 206); perhaps the function of this is to ensure that this region shall be the pace-maker of the heart.

Certainly the heart is very sensitive to asphyxiation. If the last seven pairs of spiracles in the silkworm larva are occluded, the heart generally stops.[186] Nymphs of *Cloëon* show immediate stoppage of the heart in water saturated with carbon dioxide [42]; and the pulsations in the embryo of *Melanoplus* are slowed 50 per cent. by 4 per cent. of carbon dioxide.[167]

Nervous control of the heart—The heart receives a double innervation; (i) from the paired cardiac ganglia of the stomatogastric system, (ii) from the segmental ganglia of the ventral chain [93, 190]; and in the cockroach a third supply of supposedly sensory fibres comes off the sensory branches to the dorsal body wall and reaches the heart from above.[2] All these fibres unite to form a pair of lateral nerves, which run along the sides of the heart and give off a plexus of branches ending in its muscular wall and in the alary muscles (Fig. 202).

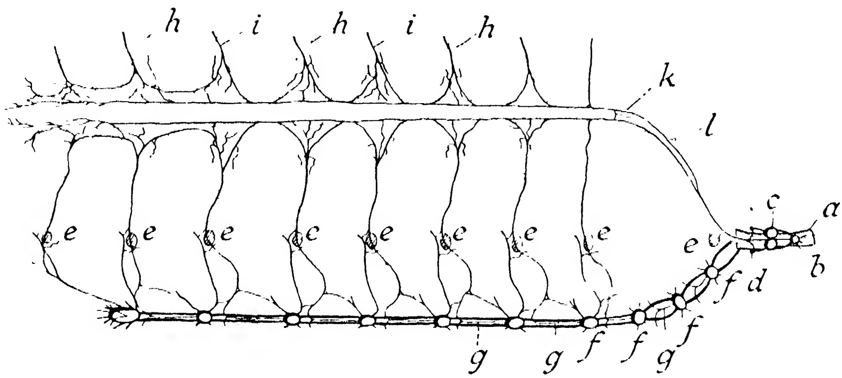


FIG. 202.—Innervation of the heart in the silkworm larva as seen in an insect dissected along the spiracular line of the right side (*after* KUWANA)

a, gut; *b*, frontal ganglion; *c*, supraoesophageal ganglion; *d*, pharyngeal ganglion of sympathetic system; *e*, spiracles; *f*, ganglia of ventral nerve cord; *g*, unpaired ventral nerve; *h*, alary muscles of heart; *i*, nerve from ventral nerve cord; *k*, dorsal vessel; *l*, lateral nerve of the heart.

Nerve cells and ganglia are usually completely absent from the heart itself [93, 103, 190]; but in the cockroach nerve cells scattered all along the lateral nerves have been described.[2]

In discussing the automatism of the heart we saw that this is probably myogenic; but the rate and amplitude of beat are under nervous control. Electrical stimulation of the brain in the grasshopper,[106] and in the larva of *Lucanus*,[98] usually arrests the heart. Faradic stimulation of the nerve cord in the neck of the cockroach after decapitation, causes immediate acceleration; the impulses reaching the heart through both the ventral nerve cord and the lateral nerves.[153] And, as we have seen, any disturbance of the intact insect will increase the rate of beat. Nothing is known of the control of the accessory hearts.

THE HAEMOLYMPH

The blood or haemolymph is a clear fluid, sometimes colourless, often tinged with green or yellow pigment. In the cockroach it forms only some 5 or 6 per cent. of the total body weight [184]; but in soft bodied larvae it comprises a quarter or a third: 25–30 per cent. in the larva of the honey-bee [11]; 20–40 per

cent. in various caterpillars.[139] It is the medium through which all the chemical exchanges between the organs are effected, hormones conveyed, food carried from the gut, and waste products to the excretory organs. It plays a certain part in respiration, which has already been discussed (p. 205), and a most important part in transmitting pressure from one region of the body to another—as in the ventilation of the tracheal system (p. 192), hatching, ecdysis, and the expansion of the wings (pp. 12, 28).

Composition of the blood : protein—In the larva of the honey-bee the total protein in the blood, 6·6 per cent., approximates to that in human plasma ; and its two chief components, 'albumen' (3·46 per cent.) and 'globulin' (3·10 per cent.) are in about the same proportions.[11] But in most insects the quantity of protein is less than this : 5 per cent. in *Bombus*, 3–4 per cent. in *Hydrophilus* and other Coleoptera, 2·6–3·3 per cent. in *Aeschna* nymphs, 1·3–2·6 per cent. in *Limnophilus* larvae (Trichopt.), 1·0 per cent. in *Dixippus*.[41] In the silkworm it rises from just over 1 per cent. in the 4th instar, to nearly 6 per cent. at the time of spinning the cocoon ; it then falls to 2·6 per cent. in the ten-day old pupa, and below 2 per cent. in the adult at emergence.[41]

Non-protein nitrogen : amino-acids—The ratio of non-protein nitrogen to total nitrogen is about 1 : 2, in the larva of the honey-bee [11] and in the mature larva of *Deilephila* (Lep.) [62] ; and of the non-protein fraction some 50–85 per cent. is in the form of amino-acids. The amino-acid nitrogen is 1·34 gm. per litre in the *Dytiscus* adult, 3·27 in the pupa of *Attacus*, 3·22 in the pupa of *Sphinx*, 2·34 in the larva of *Cossus*,[38] 1·7 in the larva of *Deilephila* [62]—values 50 or 100 times greater than the normal for mammalian plasma. In terms of amino-acid concentrations these values must be something like 20 per cent. and approach those for urea in Selachian blood.[38]

Uric acid ranges from 5·3 mg. per cent. in the larva of *Apis*,[11] 10·7–14·5 in *Hydrophilus*,[41] to 20 in the larva of *Deilephila*.[62] In the silkworm it is about 10 mg. per cent. while the larva feeds, less than 5 mg. at other times [95] ; but it may go up to more than 16 mg. per cent. in the mature larva when accumulated waste products are being discharged into the blood.[77]

Sugar and reducing substances—Another peculiarity of the insect plasma is the high content of reducing substances other than sugar. Estimations carried out on insect blood by the ordinary methods for blood sugar, yield results, expressed as glucose, of the same order as in mammals : 0·1–0·2 gm. per cent. in the silkworm,[95] 0·127 in *Deilephila*,[62] 0·203 in the larva of the honey-bee,[11] 0·02–0·104 in *Hydrophilus*.[41] But the bulk of this reducing substance is not sugar ; the fermentable reducing substance (the true blood sugar) forms only about a quarter of this figure : 0·018–0·040 gm. per cent. in different caterpillars [63] ; 0·020 in the silkworm during feeding, almost zero at other times,[94] rising to 0·049 at pupation [41] ; 0·006–0·031 in *Hydrophilus*.[41] There is, however, a striking rise in the concentration of true sugar after acid hydrolysis.[94] The nature of the other reducing substances is not known ; they show a great increase before ecdysis.[33]

Fat is present in the blood in the form of 'lipomicrons' or minute fat particles. In the cockroach these are most plentiful 14–19 hours after a meal, especially if this contains much fat.[56]

Salts—The anions and kations in the blood of insects show certain constant features. The greater part of the total osmotic pressure being made up, as we

have seen, of amino-acids, the chlorides are correspondingly reduced. In the larvae and pupae of Lepidoptera never more than 15 per cent. of the total molecular concentration is attributable to chlorides, as opposed to 65–70 per cent. in mammals.[135] In the imago of *Dytiscus*, chlorides expressed as sodium chloride are 0·37 per cent., in the larva of *Cossus* 0·04 per cent.,[135] in the larva of *Pieris brassicae* 0·16 per cent.,[14] in the bee larva 0·19 per cent.,[11] in the larva of *Culex* 0·28 per cent.[180]—as opposed to about 0·6 per cent. in mammalian plasma. On the other hand, the phosphate concentration is anything from 5 to 20 times that in mammals. In the adult of *Dytiscus* the inorganic phosphorus is 10·5 mg. per cent., in the larva of *Attacus* 12·0–15·5, in the larva of *Cossus* 11·5,[38] in the larva of *Sphinx* 66 mg. per cent.,[14] in the larva of the bee 31 mg. per cent.[11]

Of the kations, sodium seems always to be replaced largely by potassium. In the bee larva, sodium 0·012–0·017 gm. per cent., potassium 0·095 gm. per cent.[11]; in the pupa of *Sphinx pinastri*, sodium in traces only, potassium 0·138 per cent.[14]; in the larva of *Saturnia pavonia*, sodium 0·007 per cent., potassium 0·153 per cent.[35] Whereas in the plasma of mammals there is far more sodium than potassium. Calcium is rather higher than in man; 0·014 gm. per cent. in the bee larva,[11] 0·033 gm. per cent. in the pupa of *Sphinx pinastri*,[14] 0·028 in *Saturnia pavonia*.[35] And magnesium is far higher; 0·19–0·22 gm. per cent. in the bee larva [11] (about eight times the value in human blood), 0·056 gm. per cent. in the pupa of *Sphinx*,[11] 0·044 in *Deilephila*.[62]

There is an excess of fixed base in the blood. In the pupa of *Sphinx pinastri* there are 0·096 basic equivalents, against 0·046 acid equivalents; giving an excess of 0·050 gm. equivalents per litre. This excess is combined to a small extent as bicarbonate, but chiefly with proteins and organic acids.[14]

Reaction—The blood is usually very slightly acid. The *p*H in the bee larva, at the carbon dioxide tension of 35–60 mm. of mercury normal in the blood, is 6·77–6·93 (average 6·83) [11]; in *Melanoplus*, *Chortophaga*, and other grasshoppers, 6·4–7·0 (usually about 6·7) [12]; in the silkworm larva, 6·7–6·8 and in the imago 6·6–6·7 [33]; 6·2–7·2 among beetles.[41, 82]

Osmotic pressure—The total molecular concentration in insect blood is rather high. The values have usually been expressed in terms of depression of freezing point (Δ). If they are converted into concentrations of sodium chloride of the same osmotic pressure, we get instead of 0·9 per cent., the usual value in mammals, in the bee larva 1·5 per cent.,[11] in the adult *Dytiscus* 1·25 per cent. and *Hydrophilus* 1·05,[7] in various species of water beetles 0·8–1·9,[4] in the larva of *Saturnia carpini* 0·9, in the pupa of *Sphinx ligustri* 1·15,[135] in the various Ephemerid nymphs 0·69–1·0 [43] and in the larvae of mosquitos 0·75–0·89.[180]

The osmotic pressure of the colloids in the blood, which in human plasma has a value of 30–40 cm. of water, has the low value of 5 cm. in *Mantis*, the only insect studied.[114]

Regulation of osmotic pressure—If the insect struggles violently, in the absence of oxygen, the osmotic pressure of the blood is increased by metabolites set free from the muscles; and as we have seen, this change may have important effects upon respiration in the tracheoles (p. 186). In the mosquito larva *Culex* the osmotic pressure of the blood may rise during asphyxiation from 0·85 per cent. 'sodium chloride' to 1·1 per cent.[180] On readmission of air the normal level is soon restored.

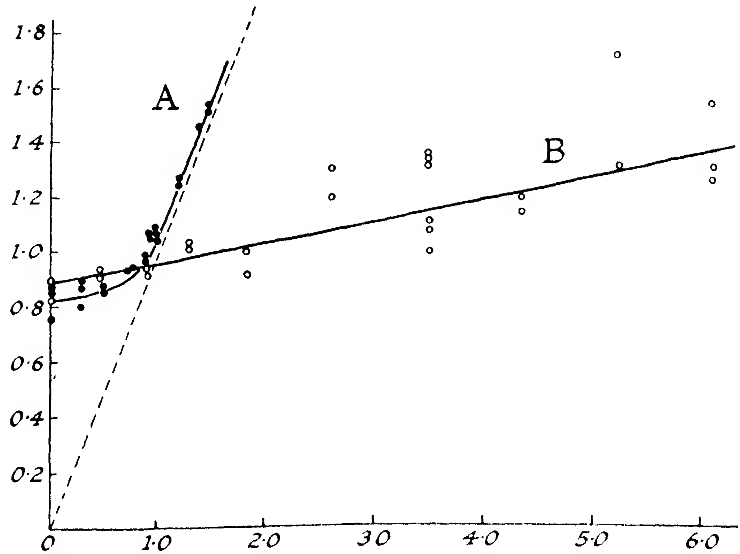


FIG. 203.—Relation between osmotic pressure of the medium and of the haemolymph in mosquito larvae

Ordinate : values in haemolymph. Abscissa : values in medium. Both expressed as equivalent concentrations of NaCl in gm. per 100 c.c. The broken line shows where the points would fall if blood and medium had the same composition. *A*, dots, larvae of *Aëdes aegypti*, which breeds normally in rain water (*after* WIGGLESWORTH) ; *B*, circles, larvae of *Aëdes detritus*, which breeds normally in brackish water (*from unpublished experiments by* L. C. BEADLE).

Mosquito larvae, *Culex pipiens* and *Aëdes aegypti*, which breed normally in fresh water, when they are reared in sea water hypertonic to the normal blood, show an increase in osmotic pressure so that the blood becomes isotonic with the external medium (Fig. 203, A). Larvae with their osmotic pressure raised in this way to the equivalent of 1·5 per cent. of sodium chloride may survive. Conversely, if the larvae are starved, the osmotic pressure falls from the normal 0·85 to about 0·7. If they are starved in chloride-free distilled water, the chloride in the blood falls from the normal level of 0·3 per cent. (expressed as NaCl) to 0·05 per cent. ; yet the total osmotic pressure is scarcely any lower (0·65–0·7) (Fig. 204). Clearly the loss of chloride has been compensated by the liberation of some other substance into the blood, perhaps amino-acids.[180] Water beetles, *Dytiscus*, immersed for 24 hours in 3 per cent. sodium chloride, show an increase in osmotic pressure in the blood from 1·2 per cent. NaCl to 2·0 per cent.[4]

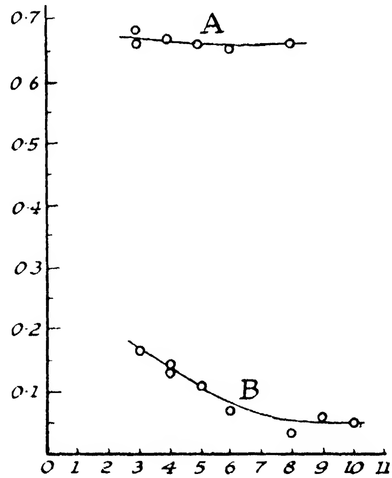


FIG. 204.—Effect of starvation in distilled water on osmotic pressure (*A*) and chloride content (*B*) in the haemolymph of the mosquito larvae (*Aëdes*) (*after* WIGGLESWORTH)

Ordinate : values expressed as equivalent concentrations of NaCl in gm. per 100 c.c. Abscissa : days after beginning of starvation.

Insects adapted to life in salt water doubtless have greater powers of regu-

lating the osmotic pressure of the blood. Thus the aquatic bug *Sigara lugubris* is a brackish water insect occurring in pools with a salt content of 0·5–1·8 per cent. Throughout this normal range the osmotic pressure of the blood remains constant at − 0·7° C. (= 1·2 per cent. NaCl); the osmotic pressure does not increase until that range is exceeded (Fig. 205). Whereas the related forms *S. distincta* and *S. fossarum* are homoiosmotic only in the fresh water which they normally inhabit.[26] And the mosquito larva *Aëdes detritus*, which breeds in salt or brackish water, is able to maintain the normal osmotic pressure in the blood, although living in a hypertonic medium, in a way that *A. aegypti* cannot. Even when in water of a salinity equivalent to 6 per cent. NaCl it is able to prevent the osmotic pressure of its blood from rising above 1·3 − 1·4 per cent. (Fig. 203, B).[8]

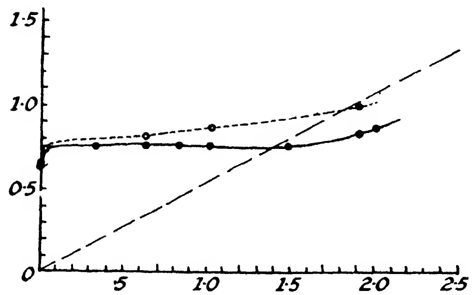


FIG. 205.—Comparison of external medium and haemolymph in *Sigara lugubris* from brackish water (continuous line), and *Sigara* species for fresh water (broken line) (*after* CLAUS)

Ordinate: Δ. Abscissa: salinity of external medium per cent. Straight line shows values of external medium.

Pigments—Haemoglobin imparts a bright red colour to some Chironomid larvae (p. 207); apart from this the blood is usually colourless, green or pale amber. In many phytophagous insects the blood is green, due, it has been supposed, to altered chlorophyll. In the larvae, pupae and imagines of Lepidoptera this green colour is more common in the female; the blood of the male is often colourless or pale yellow.[50] The significance of this difference is unknown; nor whether it is due to sexual differences in pigment absorption or in chemical metabolism [152]; chemical differences can certainly be demonstrated in the blood proteins of the two sexes of *Pieris brassicae*. In Lepidoptera the green pigment resembles chlorophyll spectroscopically; but spectroscopic tests are of doubtful value in characterizing the pigments from plants; and chemical examination of the blood of caterpillars has revealed neither chlorophyll nor xanthophyll, though carotin is present (p. 334).[115] The green blood of Phasmids shows no absorption bands like those of chlorophyll.[147]

Blood escaping from insects quickly blackens on exposure to the air; melanin being formed when the oxidizing enzymes in the blood come in contact with chromogens liberated from the injured tissues (p. 332).

'Reflex bleeding'—Certain insects when handled discharge small drops of fluid from various points of their body. Sometimes this is the product of special groups of dermal glands, as in the Mexican bean beetle (*Epilachna*) which ejects a bitter yellow fluid from the femoro-tibial articulations.[109] But in many cases the fluid is actually blood, as was originally supposed by Leydig. It appears around the mouth, the limb joints, and the base of the elytra, in such beetles as *Coccinella*, *Timarcha*, and Meloids, and the Orthoptera *Eugaster* and *Ephippiger*. Apparently the blood is strongly compressed by contraction of the abdomen, and escapes by rupture of the skin at points of least resistance.[30] In the Acridiid *Dictyophorus* the blood is mixed with air to form a brownish foam as it escapes; apparently both tracheae and body wall are perforated.[54]

But in many insects, preformed pores or ostioles exist (perhaps these mark the earlier site of glands); in the Tenthredinid larvae *Cimbex* and *Trichiosoma* these pores are closed by valves when the blood pressure is relaxed.[70] In all these forms the blood contains cantharidin, or other caustic or repellant substance; and there is evidence that the habit is of some biological value as a protection from predators.[30] On the back of *Pseudococcus*, and at the apex of the abdominal tubes or siphunculi of Aphids are ostioles, opened by a special muscle, which allow wax-laden blood cells to escape.[168]

Blood coagulation—The blood of various beetles, when collected in glass capillaries, usually clots within 5–10 minutes[82]; and clotting is particularly rapid in the vesicant species and others subject to 'reflex bleeding'.[106] On the other hand, the blood of the honey-bee larva,[11] of *Rhodnius* and some other insects, seems never to coagulate. And in many forms, such as the cockroach, the apparent coagulum is merely a clump of haemocytes; there is no gelation of the plasma.[183] The blood cells become round, develop thread-like pseudopodia, and agglutinate to form a plug which is the essential factor in the formation of the clot.[117] But in other insects, such as *Gryllus*, there is a true coagulation of the plasma with the formation of fibrin.[183] When this occurs, clotting is not prevented by oxalate; calcium is not necessary for clotting.[117]

HAEMOCYTES

The haemocyte count—The loose cells in the body cavity of insects are called haemocytes or blood cells. Most of these are normally resting on the surfaces of various organs, but some circulate freely in the blood. In the living larva of *Corethra* it is very evident that most of the haemocytes are sedentary; chiefly they lie along the outer surface of the heart, only a few are being carried through the dorsal vessel and along the body cavity by the blood stream. Cells travelling along the vessels in the wings of the cockroach often adhere to the walls and cease to move.[182] In the silkworm,[48] in *Aleurodes*,[170] and in *Rhodnius*, no haemocytes enter the heart through the ostia so that only the cell-free haemolymph can be said truly to circulate.

It is obvious, therefore, that the numbers of circulating cells must vary enormously from time to time. In the cricket *Gryllus assimilis*, figures ranging from 15,000 to 275,000 per cubic millimetre have been obtained (average 70,000), in *Periplaneta* 15,000–60,000 (average 30,000).[155] The highest values are obtained, however, after injury and haemorrhage, in insects attacked by parasites, and particularly during ecdysis, when there is a real increase in the number of haemocytes present.

Types of haemocytes—The haemocytes arise in the embryo from undifferentiated mesodermic tissue.[173] As occasion demands, both the independent cells in the blood,[9, 18] and the aggregations of cells along the heart,[29, 106] continue to multiply by mitosis throughout the life of the insect.

When the cells attach themselves to other tissues they tend to become pear-shaped, or fusiform, or to spread themselves out in a stellate manner applied as closely as possible to the surface.[177] They may thus assume an infinite variety of appearances (Fig. 206). But when floating freely in the blood they become round or oval and can then be grouped into fairly definite classes.

(i) Small cells with deeply staining cytoplasm and a nucleus which almost fills the cell. They are termed *proleucocytes*, and have been recognized in all groups of insects.[141] In Lepidoptera they are abundant only in the young larva [71]; and in all insects they are often seen undergoing mitosis. They are therefore regarded as young growing forms.

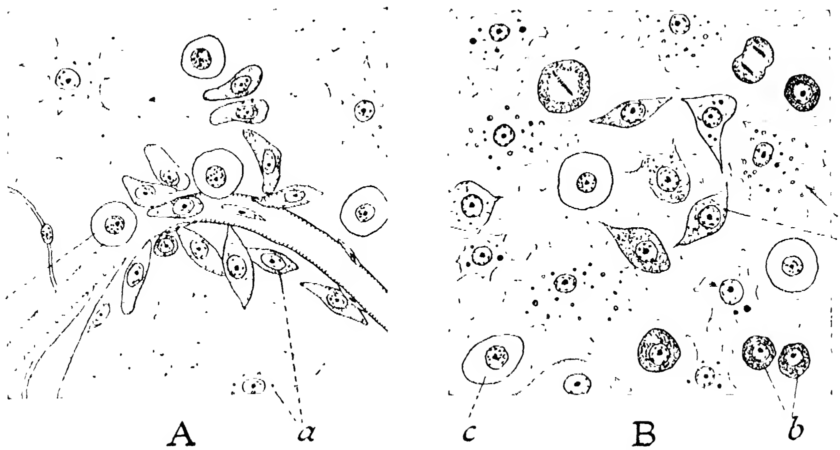


FIG. 206.—Haemocytes in *Rhodnius* (*after* WIGGLESWORTH)

A, group of haemocytes around trachea where this is passing through the basement membrane of the epidermis; a few are spread out in stellate form on the basement membrane. B, haemocytes below the basement membrane at the height of moulting; many of the phagocytes contain basophil droplets from disintegrated cells; proleucocytes dividing mitotically. a, phagocytes; b, proleucocytes; c, oenocytoids.

(ii) Rather larger cells with cytoplasm still strongly basophil and the nucleus still relatively large, showing all intermediate stages from the proleucocytes. (In the larva of the bee they are 8–11μ in diameter and the nucleus 5·7–6μ).[112] These are called *macronucleocytes*,[122] or sometimes 'chromophil leucocytes' (Fig. 207, A).[117] They also may be seen dividing.

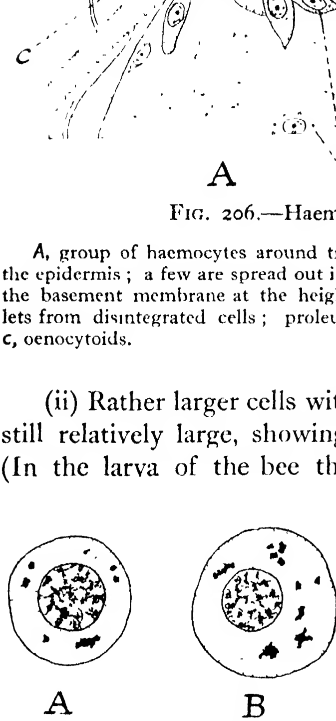


FIG. 207.—Haemocytes of the honey-bee larva after injection of Indian ink (*after* METALNIKOFF and TOUMANOFF)

A, macronucleocyte; B, micronucleocyte. Both have ingested particles of the ink.

(iii) Large cells with paler staining cytoplasm, and a nucleus smaller in proportion to the cell. (In the bee larva they are 12·7 to 15·6μ in diameter and the nucleus 5·7 to 7·1μ).[112] These are termed *micronucleocytes* [71, 122] or sometimes 'amoebocytes' (Fig. 207, B).[117]

In the bee larva the proleucocytes and macronucleocytes, treated as one class, make up 85 per cent. of the blood cells, the micronucleocytes 15 per cent.[112] In the mature larva of *Pieris brassicae* with 12,000 free blood cells per cubic millimetre, 36·5 per cent. were micronucleocytes, 41 per cent. macronucleocytes.[71] But the relative numbers of the different types depend to a great extent upon the treatment of the smears of blood, which suggests that the distinctions are not very profound.[141] In fact the earlier authors [29, 65] group the macro- and micronucleocytes together as 'phagocytes' of variable form.

(iv) *Granular leucocytes* present in Coleoptera are perhaps just phagocytes laden with granules of different sorts.[65, 177]

(v) *Spherule cells* with cytoplasm filled with large round inclusions appear in the blood of beetles towards pupation [65, 136]; similar cells filled with granules and vacuoles occur in Lepidoptera at metamorphosis,[65] forming about 4 per cent. of the total free cells in *Pieris brassicae* [71]; but there seems little reason to doubt that these also are derived from the phagocytes.[73]

(vi) *Adipoleuocytes* are cells ranging from 12–35μ, filled with droplets of fat, observed in *Pyrrhocoris apterus.*[65] They are said eventually to settle down and form a tissue resembling fat body. Similar cells laden with wax are present in the blood of Coccids and Aphids.[65]

(vii) *Oenocytoids* are round or oval cells, 8–12μ in diameter, with darkly staining nucleus and a uniform acidophil cytoplasm. They are present in Coleoptera,[136] Hemiptera (Fig. 206),[65, 177] Lepidoptera [111] (they form 3 per cent. of the circulating cells in *Pieris* larvae [71]), but are wanting in Orthoptera [29, 65] and perhaps in Hymenoptera.[112] These cells seem quite distinct from the proleucocytes and phagocytes (i–iv); they have been thought to be derived from the true oenocytes (p. 241) [131] but in the bug *Rhodnius* that is certainly not the case.[177]

Phagocytosis and phagocytic organs—The most obvious activity of the haemocytes is phagocytosis or the ingestion of small solid particles. They will take up injected particles of Indian ink or carmine [29] or dead bacilli [111] or the dead bodies of one another [65, 177] or the histolysing tissues in moulting and metamorphosis.[127, 136] In the Thysanura all the corpuscles appear to be phagocytic [18]; but usually the proleucocytes are not [29, 65, 177]; the oenocytoids when present never are.[177] The most active phagocytes are the micronucleocytes and to a less extent the macronucleocytes.[71, 112]

The sedentary blood cells tend to accumulate along the dorsal vessel. In Gryllidae, Acridiidae, *Forficula*, they form dense aggregations, 'phagocytic organs', through which the blood must work its way as it passes to the heart.[29] These organs are devoid of tracheae; they differ from accidental accumulations of haemocytes only in the constancy of their position and form. They have the same properties as the free cells in the blood, multiplying by mitosis and ingesting foreign particles. In Blattids, Mantids and Locustids, indeed in most insects, compact phagocytic organs are wanting; there are only simple groups of phagocytes around the pericardial cells and in the interstices of the pericardial septum.[29] In Thysanura the pericardial septum seems itself to be composed of a syncytium of phagocytic cells.[18, 130]

The transition from free haemocytes to phagocytic tissue is well seen in Chironomid larvae. In *Tanypus*, haemocytes alone are present; in *Chironomus plumosus* and *lobiferus*, circulating cells are entirely wanting, being replaced by a reticular phagocytic tissue at the hind end of the abdomen, through which the blood must pass to reach the heart; in *Tanytarsus roseiventris* free cells and tissue are both present.[96]

Functions of haemocytes—Under normal circumstances the chief function of the haemocytes appears to be the removal of the larger particles of solid matter set free into the body cavity. Hence they increase enormously in numbers during moulting (Fig. 206, B) [131, 177] and metamorphosis [127, 136]; and at these times they tend to leave their fixed stations along the heart and

become scattered throughout the body.[116] We have seen that the histolysis of tissues may be achieved completely without the intervention of the blood cells; but that, in many insects, once degeneration has set in, they play a great part in removing the dying cells and tissue residues [20] (p. 42). The activity of the haemocytes in removing debris from the haemolymph at the time of moulting is well seen in the bed-bug *Cimex*. Sometimes, after a meal of blood, the stomach of this insect may rupture and red corpuscles be set free into the body cavity. Here they may persist unaltered for several weeks [176]; but if the insect moults they disappear completely.[81] In the later stages of moulting [177] and metamorphosis [136] the excess haemocytes themselves decay and are ingested by their fellows. The oenocytoids, which play no part in phagocytosis, show the same proliferation during moulting and metamorphosis and suffer the same fate when the process is complete.[136, 177] Their function is not known.

The haemocytes may thus be pictured as removing from the blood the grosser particles of suspended matter. The products of digestion are presumably returned to the blood in solution, and taken up by the tissues. But nothing is known of the stages in this process.

In the same way, haemocytes collect at the site of injuries, forming a plug which helps to seal the wound, proliferating, and removing the dead and discarded cells.[140, 179] They ingest small particles such as Indian ink or carmine or bacteria, especially dead bacteria, and forms like tubercle bacilli which are not pathogenic to the insect.[111] And if such particles are not digestible, the cells containing them fuse into plasmodial aggregates, and become surrounded and walled off by other cells to form small 'tubercles' [29] in which the central parts break down while fresh cells congregate at the periphery.[111] They collect round foreign bodies to form a capsule [99] (though other cells such as the fixed cells of the tracheal tissue may also take part in this [75]). In the larvae of Lepidoptera, the eggs and young larvae of Hymenopterous parasites become encysted by blood cells; such parasites induce mitosis and capsule formation [13] (though around the larger larvae of parasites, blood cells may be absent altogether [29]). Trematodes in *Dytiscus*,[68] and in the flea,[24] become invested in a thick mantle of haemocytes. Usually the cellular character of these capsules persists; but sometimes the cell bodies are converted into fibrous sheets and strands, the nuclei disappear and a capsule of connective tissue is produced.

Connective tissue—Many of the organs of insects, the fat body or ovaries for example, are enclosed in connective tissue membranes devoid of fixed cells, and consisting of networks of branched elastic fibres in a homogeneous ground substance.[99] Some authors regard these membranes as the product of the anastomosing tracheal end cells (p. 184); others believe them to be formed, like the connective tissue capsules around foreign bodies, from the wandering blood cells which have flattened themselves upon the surface, fused by their processes into a syncytium, and lost their nuclei by degeneration.[99] The same origin has been suggested for the basement membrane of the epidermis.

Giant cell formation—Giant cells may appear around the sites of chronic inflammation (around foreign bodies or slowly healing wounds), originating in two ways. (i) By the fusion of haemocytes or other cells into multinucleate masses [75]; (ii) by the hypertrophy of single cells. Giant cells of the second

type, ranging up to 150μ in diameter, have been termed ' teratocytes ' and regarded as hypertrophied macronucleocytes.[71] But it is certain that the cells of many other tissues, epidermis,[177] fat body, Malpighian tubes, gonads, can suffer the same change in the proximity of internal parasites.

Immunity—The natural immunity of insects to bacteria is chiefly a phagocytic immunity.[25] The numerous bacteria in the gut of *Calliphora* larvae are liberated into the body cavity of the pupa during histolysis. But these are actively destroyed by the phagocytes ; so that at the time of emergence, or within a few hours afterwards, the adult flies are bacteriologically sterile.[5] Non-pathogenic organisms such as tubercle bacilli, or feebly pathogenic forms such as staphylococci, are rapidly taken up by phagocytes of *Galleria* and other caterpillars [111] ; whereas the pathogenic coccobacilli, &c. are not.[71] Acquired immunity, resulting from infection with pathogenic organisms, is a general immunity in which the essential response seems to be an increased sensitivity towards the organism on the part of the body cells ; the nervous system being apparently connected in some way with the change.[111] Phagocytosis, giant cell formation, and encapsulation, are more active. And various antibodies are produced : agglutinins for the pathogenic *Coccobacillus acridiorum* appear in the blood of immune *Melanoplus* [51] ; bacteriolysins, apparently non-specific, can be evoked by many organisms or even, it is said, by injections of foreign proteins [191] ; specific antitoxins can be formed.[25]

It is well known that entomophagous parasites show some specificity as regards the hosts in which they will develop. The caterpillars of *Loxostege sticticalis* are non-susceptible to *Eulimneria* spp. (Hym.). This type of immunity is due to the very active encapsulation by the blood and tissue cells.[97]

PERICARDIAL CELLS AND SO-CALLED ' NEPHROCYTES '

Anatomy and Histology—The pericardial cells which were recognized by Leydig in 1866 are of mesodermic origin like the haemocytes. But they are incapable of migrating in the blood stream, and occupy fairly constant positions. In *Machilis* they resemble fat cells, and lie along the borders of the connective tissue around the pericardial sinus ; in *Lepisma* they are suspended on the fibres which attach the heart to the dorsal body wall.[18] They are usually arranged upon the surface of the heart ; often they are scattered also over the pericardial septum and the alary muscles ; in Hemiptera-Heteroptera they are very abundant and lie inside the heart as well (Fig. 192, *c*). They may be large, few and isolated, as in the larvae of mosquitos ; or small, numerous and closely packed.[17] The fully formed cells usually contain more than one nucleus [69] ; in *Galleria* larvae they are large syncytia containing as many as six nuclei of variable size.[111] They often contain vacuoles and granular inclusions, which vary in size from time to time ; sometimes they are filled with yellow, brown, red or green pigments. In Lepidoptera, the cells show peripheral striations.[69]

Function of pericardial cells—The most obvious property of the pericardial cells is their ability to absorb colloidal particles from the blood. Haemoglobin, chlorophyll, egg-white, trypan blue, ammonia carmine, injected into the blood, appear as inclusions in the pericardial cells.[69, 101] When they take up litmus these cells are always red or acid [86] ; and when ammonia carmine

is absorbed it is believed that this acidity causes the liberation of the insoluble free carmine [69] with the result that the dye persists indefinitely in the cells. Indeed when the pericardial cells become completely saturated with the dye, they break down and are destroyed by phagocytes (in Muscid larvae [86] and *Galleria* larvae [111]).

Injection of ammonia carmine reveals that there are other cells with similar properties in the insect body. Cells scattered through the fat body in *Lepisma*, Odonata nymphs [17] and *Pediculus* [120]; in groups around the mouth appendages or at the bases of the limbs in *Gryllus*, *Mantis*, *Periplaneta*,[19] or on either side of the oesophagus in *Pediculus* [120]; and forming a garland of cells that hangs between the salivary glands of Muscid larvae.[79]

All these clearly belong to the same system as the pericardial cells. The whole are often classed as 'nephrocytes' in the belief that the accumulation of injected dyes within them proves that their function is the segregation and storage of waste products.[86] But with few exceptions (p. 319) the contents of these cells do not increase with age [29]; and if they play a part in excretion it is likely to be rather in the intermediary metabolism of waste substances.[29, 69]

The current view on this system is that it is analogous with the reticulo-endothelial system of vertebrates [133]; that as the haemocytes are concerned in the removal of gross fragments from the blood, so these cells are responsible for the 'micro-phagocytosis' of colloidal particles—the absorption of a given particle being determined partly by its charge (electronegative colloids such as acid dyes being most readily seized), partly by the size of particle.[101] But other undetermined physico-chemical factors can influence absorption; for the uptake of dyes is somewhat capricious in different species,[69] and it may be influenced by whether the dye is injected or given by the mouth.[55] At the time of metamorphosis (in Coleoptera, Lepidoptera, Trichoptera) albuminoid inclusions appear within the cells [69, 122]—as was to be expected if they are freeing the blood of foreign colloids. Whether they liberate the products of their activity in simpler form, or whether, as has been suggested,[69] they produce the specific proteins of the blood, is quite unknown.

THE FAT BODY

Anatomy and Histology—The fat body is a more definite organ than the other tissues of the body cavity. It is derived from the mesoderm of the walls of the coelomic cavities; but its original segmental arrangement is generally lost when the embryonic coelom breaks down; so that the fat body is arranged as a loose meshwork of lobes, invested in delicate connective tissue membranes (p. 236), and joined by connective tissue strands, so as to expose the maximum of surface to the blood. In spite of the looseness of its texture, the fat body is arranged in a constant manner in each species. Often there is a peripheral layer beneath the skin, and a central layer as a sheath round the gut, but in many insects the peripheral part alone is present. There may also be cylindrical cords in the thorax, compact masses in the head, and so on.[73, 90, 175] Though present in all insects, the fat body is most conspicuous in the larvae of holometabolic forms; in the full-grown larva of the honey-bee it makes up 65 per cent. of the total body weight.[10]

In the embryo and newly hatched insect the cells are in loose strings;

they are round, with a homogeneous chromophil cytoplasm, free from vacuoles or inclusions.[90, 131, 136, 145] When the larva starts to feed the cells become vacuolated and increase in size (Figs. 208, 209). At moulting, they multiply by mitosis and gradually become so large and distended that the cell boundaries are no longer visible. But they do not actually form a syncytium; for in the larva of *Dytiscus*, after prolonged starving, the cell limits reappear; vacuoles and granules disappear completely, and the cells revert to their embryonic form [90]; and when the larva of the honey-bee becomes full-grown, the cell boundaries can again be seen.[145]

Relation to the blood cells—In the very young insect the fat body cells are scarcely distinguishable from the haemocytes; and the relations between these two tissues have often been considered to be very close.[99] What appear to be ordinary haemocytes enter the histolysing flight muscles of queen ants and become converted into fat cells; and the imaginal fat body of Muscids arises from 'mesodermal leucocytes' in the same way.[76] In the Coccinellid *Harmonia*, blood cells enter the elytra and later become vacuolated and adhere together to form fat body.[88, 89] In aquatic Hemiptera, the fat body is said to increase in size throughout life by the inclusion in its substance of free adipocytes, that is, haemocytes charged with fat (p. 235).[131] And in *Aleurodes*, since all the fat cells are freely floating in the body cavity, it is impossible to draw any line of distinction between them and the haemocytes.[170] In Lepidoptera,[73] and Coleoptera [116, 136] the fat cells are said to be capable of phagocytosis and to ingest the debris of histolysed tissues; but most of the formed elements appearing in the fat body during metamorphosis are undoubtedly taken up from the blood in solution.[128]

Functions of the fat body during growth—The fat body may have other functions in intermediary metabolism (p. 317) but its most obvious purpose is the storage of reserve materials. 'Fat body' is a misnomer, for it is equally important as a store for proteins and sometimes for glycogen. Fat droplets and protein granules usually appear soon after feeding has begun. If the larva is starved, the cells become completely emptied (*Dytiscus*,[90] *Tenebrio* [84]), to fill again when feeding is resumed. Towards the end of larval life the cells are filled with drops of fat, spheres of protein, and glycogen in granules or diffused through the cytoplasm. In the fat body of the silkworm the glycogen is estimated to range from 2–17 per cent.[187]; in the mature larva of the bee it forms more than 33 per cent. of the dry weight of the whole insect.[149] The same accumulations appear in the young stages of hemimetabolic forms.[131]

These reserves may be used up to some extent during moulting (in *Galerucella* [136]) but it is at metamorphosis that they are chiefly called upon.[9]

The fat body during metamorphosis—(Figs. 208, 209)—At the approach of metamorphosis, the fat cells become intensely active. They are now stuffed with reserves. The nuclear surface is commonly increased by amitosis,[118] or by the elongation or branching of the nucleus.[10, 127] The nuclear membrane may become indefinite, and small basophil granules appear around it. These are variously regarded as chromatoid granules discharged from the nucleus,[10] comparable with the chromidia of the oocyte,[89] or as transformed mitochondria.[121] Gradually they spread outwards from the nucleus, increase in size, and stain less deeply with basic dyes [10] or become frankly eosinophil.[145]

As these 'albuminoid spheres' enlarge, reaching a diameter of 12–15μ in the pupa of the bee,[145] the fat droplets diminish, and the spheres begin to stain with osmic acid.[10] This whole process, as observed in the bee,[10, 145] *Dytiscus*,[90] Muscids,[127] Tenthredinids[144] and Lepidoptera,[66, 73] gives the impression that the free fat in the cells is being incorporated with the protein elements

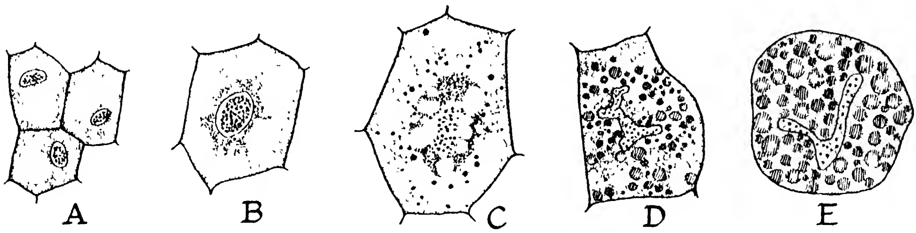


FIG. 208.—Changes in fat body cells during growth and metamorphosis in the honey-bee (*after* BISHOP)

A, shortly after hatching; cells with one large fat-vacuole. *B*, in the growing larva; cells with peripheral ring of fat-vacuoles, central densely staining cytoplasmic zone, and densely granular nucleus. *C*, in larva just becoming quiescent; central ring of fat-vacuoles indenting the nuclear vesicle, nuclear membrane absent, and nuclear granules dispersed into the cytoplasm. *D*, in early pupa; nuclear membrane reformed, fat-vacuoles disappearing, nuclear granules growing to albuminoid globules. *E*, in the middle of pupal development; cell about to disintegrate, albuminoid globules matured, their centres acidophile, fat almost absent.

to produce a raw material comparable with the yolk spheres of the egg.[89, 128] The appearance of 'pseudonuclei' of uric acid within the albuminoid spheres of Muscids (Fig. 209) is discussed elsewhere (p. 319).

During metamorphosis and moulting, these reserve materials are liberated into the blood; the cells for the most part survive.[9] But in some Hymenop-

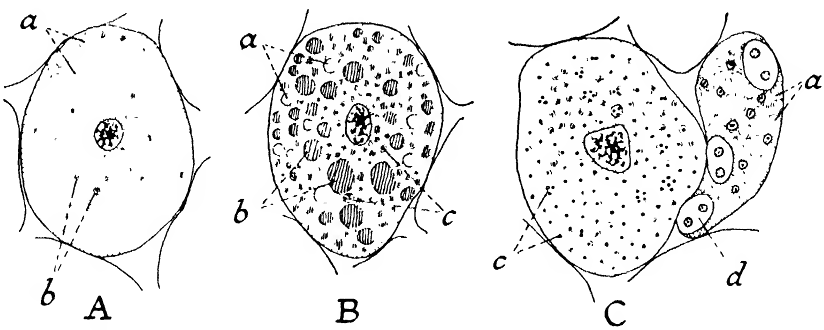


FIG. 209.—Fat body cells of *Calliphora* at different stages of growth (*after* PÉREZ)

A, growing larva; much fat, little albumen. *B*, early in pupal development; little fat, many albuminoid spheres of all sizes, some with pseudo-nuclei of uric acid. *C*, shortly before emergence from pupa. On left, remains of larval fat body, with no fat and all albuminoid spheres full of pseudo-nuclei. On right, imaginal fat body with oenocytes. *a*, fat globules; *b*, albuminoid spheres; *c*, pseudo-nuclei; *d*, oenocytes.

tera, and in the higher Diptera, there is an almost complete dissolution of the fat body in the pupa or young imago; it is then formed anew, from a few surviving cells in the bee,[145] or from embryonic cells in the Muscids.[127]

In the honey-bee, shortly before pupation, the fat cells, now filled with eosinophil inclusions, are set free into the blood, which becomes a thick creamy liquid. In the young pupa these fragile cells begin to disintegrate spon-

taneously. Fat droplets and albuminoid spheres float freely in the blood. As time goes on few intact cells remain; the albuminoid spheres, and to a less extent the oil drops, are removed; and by the time of emergence almost all the cells have disappeared. In the adult, the detritus is all dissolved, and to some extent ingested by the phagocytes, so that the blood becomes quite clear again.[149]

The fat body in the imago—Metamorphosis so far as it concerns the fat body is often still uncompleted when the adult emerges. The body cavity of newly hatched Muscid flies is full of little floating spheres which are the remains of the larval fat body. These are laden with protein granules, which disappear during egg development; they are then attacked by haemocytes.[40] In the young adult of *Culex*, the abdomen still contains the residue of larval muscles; in a few days after emergence these disappear, and the fat body (or the ovaries) grows at their expense.[143] Thus in the adult insect the fat body provides reserve material for egg production; it is often more prominent in the female.[90] It also furnishes the reserves for hibernation; in September and October overwintering females of *Culex* with a wet weight of 3 mg. contain 0·91 mg. of fat; in March and April with a wet weight of 2 mg. they have a fat content of 0·13 mg.[23]

So-called ' tracheal cells '—During the 1st instar in the parasitic larva of *Gastrophilus*, when the cells in the anterior part of the fat body begin to increase in size and accumulate fat, those in the hind part become specialized to form the ' *organe rouge* '.[34] The cells of this organ are pyriform and richly supplied with tracheae and intracellular tracheoles, and their cytoplasm contains a red pigment identical with the haemoglobin of vertebrates.[137, 138] They are often termed ' tracheal cells ' and it has been suggested, but without experimental proof, that the haemoglobin may be connected with intracellular oxidations.[80] Somewhat similar very large ' tracheal cells ', sometimes as much as 120μ in length, abundantly supplied with tracheae and containing haemoglobin, occur in the aquatic bugs *Buenoa* [74] and *Anisops*.[132] But they are absent in the closely related genera *Plea*, *Notonecta*, *Corixa*, &c. For this reason it has been thought that haemoglobin arises in these cells as an accidental product of metabolism without functional significance.[132]

THE OENOCYTES

Anatomy—Unlike the other tissues of the haemocoele, which are of mesodermal origin, the oenocytes appear early in embryonic development by the enlargement of metameric groups of ectodermal cells. With the exception of the Thysanura *Lepisma*, *Campodea*, *Anurida*,[173] oenocytes, which were discovered originally by Fabre, are universal among insects. In many forms their segmental arrangement is retained: in Ephemeroptera, Odonata, Plecoptera, and Termites, they occur as clumps in the pleural epidermis near the spiracles [173]; in Lepidoptera they are in close relation with the spiracular tracheae [154]; in the Tachinid larva *Thrixion* they form groups of 3–6 large cells, strictly metameric, between the muscles and the body wall.[123] But often they are dispersed at random. They may remain in close relation with the epidermis: they lie scattered between the epidermis and the basement membrane in *Tomocerus* (Collembola),[181] *Blatta*,[173] Phasmids,[147] *Melolontha*,[175]

Hemiptera-Heteroptera.[131, 175, 177] But often they tend to lose this primitive connexion: in the larvae of the beetles *Galerucella*,[1] *Melasoma*, *Harmonia*,[89] and *Calandra*,[116] though they are massed particularly in the neighbourhood of the spiracles, they extend inwards and merge with the fat body; in *Dytiscus* larva they form long bands between the tracheal trunks [90]; in *Geotrupes* (Col.),[175] Aphids,[173] Hymenoptera (Fig. 210),[85, 127, 145] adult Muscids,[127] &c. they are scattered throughout the fat body.

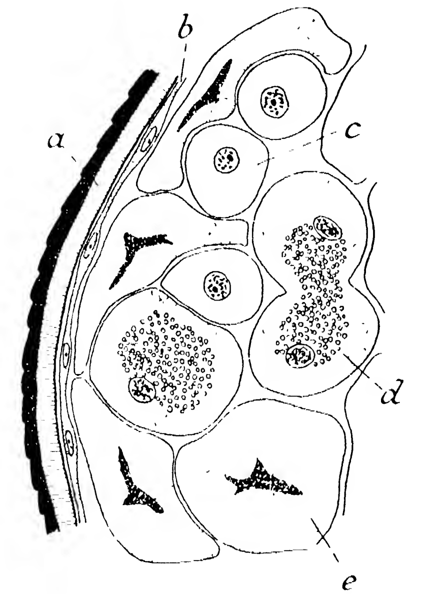


FIG. 210.—Section through dorsal region of the abdomen in the ant *Tapinoma* to show the underlying tissues (*after* BERLESE)

a, cuticle; *b*, epidermis; *c*, oenocytes; *d*, urate cells with uric acid concretions; *e*, ordinary fat body cells.

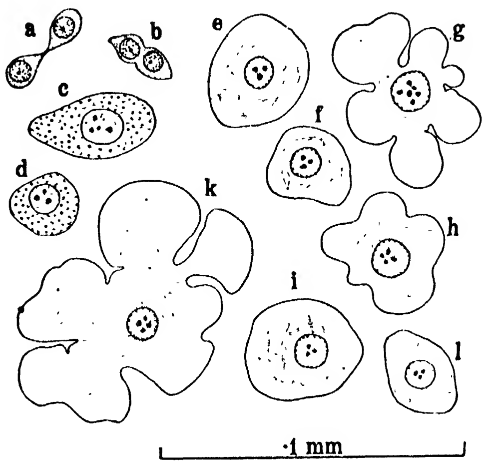


FIG. 211.—Oenocytes in *Rhodnius* (*after* WIGGLESWORTH)

a, *b*, young forms; *c*, *d*, resting forms with granular inclusions; *e*, *f*, forms with spindle-shaped clefts; *g*, *k*, forms at height of secretory activity; *h*, *i*, forms with needle-like crystals in cytoplasm.

Histology.—In the fresh state the oenocytes are often of a wine-yellow colour to which they owe their name,[175] but in many insects they are colourless. Usually they are large cells few in number: in some Cynipid larvae they are 150μ across, one-fifth of the whole length of the insect [142]; in the pupa of the queen bee 176μ [85]; quite commonly they are 60–100μ. In adult insects they tend to be more numerous and small (15–25μ in mosquitos,[72] 8–30μ in *Melasoma* (Col.) [89]). They adhere closely to one another, or to the fat body (in Muscid flies they are completely buried in the fat cells [127]) but they are not usually bound by tracheae or connective tissue. They are often lobulated, and certainly seem to migrate about the body; but they have not been seen to make amoeboid movements in the living state.[67] In stained preparations the oenocytes are characterized by their dense, eosinophil and usually homogeneous cytoplasm; but sometimes this is granular or vacuolated, and in fixed material it may show spindle-shaped clefts (which may be artefacts [123, 177]), or radiating canals [90, 154]; and sometimes it contains elongated apparently crystalline bodies best seen in fresh preparations [67, 177]; and granules of pigment of many kinds may occur.[67] (Fig. 211).

Generations of oenocytes—In the aquatic Hemiptera, fresh oenocytes continue to arise from small subepidermal cells throughout post-embryonic life [131]; in *Rhodnius* (Hem.) a new generation arises in this way during every moult except the last.[177] In *Rhodnius* they show no further multiplication once they are developed, but it has been claimed that in *Gerris* they produce the oenocytoids (p. 235) by a process of budding.[131] In holometabolic insects there is only one generation throughout larval life (larval oenocytes); a second generation arises at pupation (imaginal oenocytes).

Larval oenocytes—In *Ephestia* (Lep.), when newly hatched, there are one or two cells round each trachea; these increase by amitosis to about 10 cells [154]; in *Bombyx mori*, on the other hand, no such increase occurs.[163] In Cynipids the larval oenocytes multiply by amitosis [142]; while in *Hyponomeuta* (Lep.) they are said to bud off small amoebocytes.[73] In *Galerucella* the larval oenocytes contain two or three nuclei, suggesting amitosis [136]; but they show no increase in *Dytiscus* [90] or *Polistes* (Hym.) [127]; and in Nematocerous larvae (*Chironomus*, *Culex*, &c.) the same 5 cells at each side of the abdominal segments, persist until pupation.[72, 175]

Imaginal oenocytes—With the possible exception of the ant *Formica* [127] and the beetle *Galerucella*,[136] in which they are said to arise by a process of budding from the larval cells, the oenocytes of the imago always develop, like the oenocytes in the embryo, from segmental groups of cells in the epidermis of the abdomen, usually around the spiracles. Metameric thickenings or imaginal discs of this kind are described in the ant *Lasius*,[78] *Apis*,[85] *Polistes*,[127] the Chalcid *Torymus*,[171] and Cynipids [142] among Hymenoptera; in *Hyponomeuta*,[73] the silkworm,[164] and *Ephestia* [154] among Lepidoptera; and in *Dytiscus* [90] and *Calandra* [116] among Coleoptera. At first they are enclosed beneath the basement membrane, but later this is broken through and they are scattered throughout the fat body. Apart from their smaller size, the imaginal oenocytes resemble those of the larva; but in *Chironomidae* they have been regarded as distinctive organs, the ‘synoenocytes’.[189]

Changes in oenocytes during growth—In the larva of *Thrixion* (Tachinidae) the oenocytes increase progressively in size: 15μ in the 1st instar, 45μ in the 2nd, 85μ in the 3rd.[123] But in some insects they undergo a cycle within each instar. In the silkworm and in *Ephestia* they increase enormously in size before moulting; the vesicular nucleus contracts and becomes branched and distorted, and the cytoplasm fills with vacuoles; as these vacuoles disappear the nucleus regains its normal form.[154, 164] A similar cycle coincident with the moulting cycle occurs in *Galerucella* (Col.),[1] and probably in aquatic Hemiptera, in which the oenocytes are small after each moult.[131] This cycle is particularly evident in *Rhodnius*: the cells swell and throw out lobes like pseudopodia while the epidermis is growing; they reach a maximum just before the new cuticle is laid down; then they shrink, many of the older generation undergoing chromatolysis and disappearing, to be replaced by the new generation that has arisen (Fig. 212).[177] The same cycle occurs in the embryo of Lepidoptera before hatching,[154] and in *Rhodnius* in the egg before the embryonic cuticle is shed.[110]

During pupal development the oenocytes show similar changes. Larval and imaginal oenocytes hypertrophy enormously; then they diminish, and many of the larval cells disintegrate, only a few surviving in the adult. A

cycle of this kind has been observed in Trichoptera,[47] Lepidoptera,[73, 154] Hymenoptera,[78, 85, 127, 142] Coleoptera [89, 136] (in *Dytiscus* the surviving cells diminish from 68μ to 25μ in the course of pupal development [90]) Diptera Nematocera,[72] and Cyclorrhapha.[127]

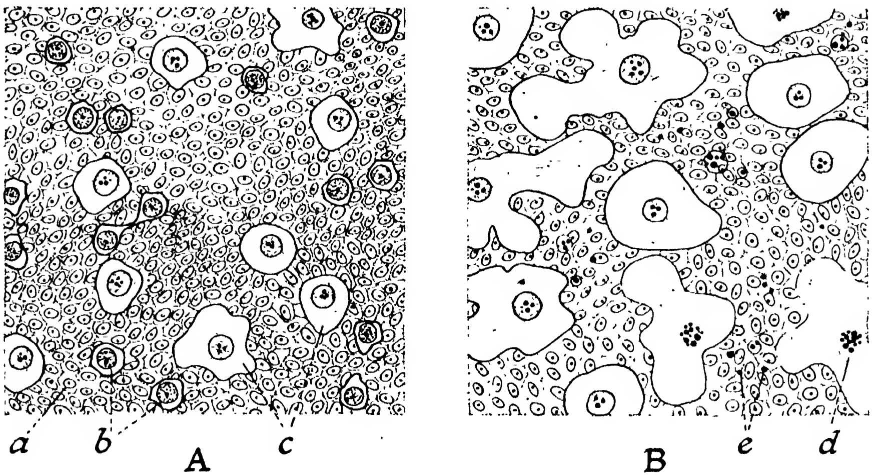


FIG. 212.—Surface view of epidermis in *Rhodnius* showing oenocytes

A, in nymph before feeding; *B*, in nymph at height of moulting process, shortly before new cuticle is id down. *a*, epidermis; *b*, new generation of oenocytes; *c*, preceding generation of oenocytes; *d*, oenocyte undergoing cytolysis; *e*, chromatin droplets derived from broken down oenocytes and other cells.

Oenocytes in the adult—Oenocytes always persist in the adult. In *Dytiscus* they increase in size again during the weeks following emergence.[90] In aquatic Hemiptera they show signs of secretory changes in the female at the times of oviposition [131]; and this is very obvious in the blood-sucking bug *Rhodnius*, in which they swell up enormously in the female after a meal, when eggs are being developed, but show comparatively little increase in the male.[177]

Function of oenocytes—Most authors are agreed that the oenocytes are organs of intermediary metabolism which discharge their secretion into the blood [67, 89]; but there is little to indicate what the nature of this secretion may be. Glycogen [67, 131] as well as protein and, occasionally, fat have been demonstrated in the cells, and their needle-like crystals have been thought to be wax.[67] The oenocytes may hypertrophy in larvae invaded by parasites,[13] and in Aphids during the lysis of their symbionts, for which they have indeed been held responsible.[161] They may accumulate pigments in old insects [85] and absorb certain dyes injected into the blood, and have therefore been thought to remove waste products or toxins from the blood, or generally to regulate its physico-chemical composition.[131]

But it is evident that the oenocytes bear some special relation with growth, and perhaps with reproduction. Possibly they secrete enzymes which further cytolysis [3] or dissolve stored substances in the fat cells.[159] Perhaps they produce the hormones which initiate moulting.[83] Certainly in the silkworm they are the first cells to show activity during the moulting cycle, while they show no such changes in a strain of silkworm which fails to moult [188]; and in Chironomidae their activity runs parallel with the development of the imaginal

..s.[189] But in *Rhodnius* the oenocytes do not reach the height of their activity until long after the moulting cycle has begun (p. 38). Their secretory changes reach their peak just when the new cuticle is being laid down; perhaps they are contributing some of the substances which go to form the cuticle.[177, 181] The imaginal oenocytes seem to be related to development of the gonads; possibly through the secretion of hormones,[189] possibly through the production of materials that go to form the eggs or their shells.[88, 177]

LIGHT-PRODUCING ORGANS

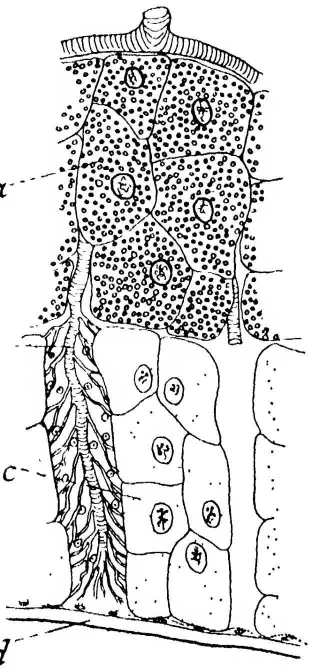
Luminescence occurs in many insects; notably Collembola, the larvae of some Nematocera, and the larvae and imagines of certain beetles, mostly Malacodermata and Elateridae. In origin it was probably an accidental accompaniment of some specific process in metabolism (for many substances slowly oxidized at low temperature in the dark are luminous) and only secondarily and in some instances has the light itself become of biological importance.[105] In many insects, such as caterpillars of Lepidoptera,[129] occasional examples of luminosity are the result of an abnormal infection with luminous bacteria [31]; but insects which show the phenomenon constantly generate the light in the course of their own metabolism.[106]

Nature of photogenic organs—A general distribution of luminescence throughout the body, except the legs and antennae, is seen in the Collembolon *Anurida* sp. which gives out a continuous pale greenish glow [6] and in *Onychiurus* (*Lipura*) *armatus* [61] and *Achorutes muscorum*,[151] also Collembola. In these insects it seems to be the fat body that is the source of the light; and that is certainly the case in the Mycetophilid *Ceroplatus testaceus*, in which the peripheral layer and the anterior part of the visceral layer of the fat body in the larva gives out a very faint persistent light. The light in *Ceroplatus* is still present in the pupa but disappears soon after the adult emerges; that is, at the time when the cells of the larval fat body dissolve.[150]

The photogenic organs of beetles are more specialized; and they are of undoubted importance, for they are usually more active in the female, and clearly provide the main stimulus in the meeting of the sexes. In Lampyrids they lie on the ventral aspect of the posterior abdominal segments; in the Elaterids *Pyrophorus*, &c. there is a pair at the posterior angles of the prothorax on the dorsal surface, and a third which lies mid ventral on the first abdominal segment. The histological details vary in different forms, but the organ consists in general of a deep layer of 'reflector cells', their cytoplasm stuffed with uratic granules, a more superficial mass of large photogenic cells richly supplied with nerves and tracheae and tracheoles (the latter often arising within conspicuous tracheal end cells (p. 184)) and a translucent unpigmented window in the overlying cuticle (Fig. 213).[31, 64] These organs, also, are probably derived, in the course of development, from the fat body [31]; for in *Lampyris*, the luminous organ of the adult beetle is certainly produced by modification of fat body cells already existing in the larva.[166] In this same insect, and in *Pyrophorus*, the female often shows a diffuse luminosity throughout the abdomen which is caused by the generation of light in the yolk of the unfertilized eggs.[36]

An unusual type of photogenic organ exists in the larva of the Mycetophilid *Bolitophila luminosa*, in which the distal ends of the four long coiled Malpighian

tubes are converted into thick rod-shaped light producing structures, and rest upon a mass of modified connective tissue (derived perhaps from tracheal cells) filled with reflecting granules.[174]



a, *b*, *c*, *d*

FIG. 213.—Section of a small part of the photogenic organ of *Luciola italica* (*after* BERLESE)

a, opaque reflector layer; *b*, tracheae; *c*, photogenic cells; *d*, translucent cuticle.

It was suggested by Dubois[36] that the production of light in many animals is brought about by luminous bacteria which maintain a symbiotic existence within the cells.[21] In the case of insects, the photogenic cells are in fact filled with uniform granules which bear a resemblance to micro-organisms: in the male of *Photinus* the granules are round like cocci, in the female they are rod-shaped like bacilli.[31] But if the luminous organ is excised from the larva of *Photuris*, although the usual glowing of the pupa is eliminated, yet perfectly normal photogenic organs develop in the adult. Unless, therefore, it be supposed that the bacteria go through a developmental stage in which they are not luminous, it must be concluded that micro-organisms are not responsible for the light production.[60]

Chemistry and physics of light production—It is generally agreed that the production of light accompanies an oxidative process in which a substrate, called by Dubois 'luciferin', is oxidized in the presence of an enzyme 'luciferase'. Luciferin can be oxidized by other agents, such as potassium ferricyanide, but no light is then produced. It is therefore believed that it is the enzyme and not the substrate which gives out the light. The process may be pictured as taking place in three stages:

(i) Luciferin + $\frac{1}{2}O_2 \rightarrow$ oxyluciferin′ + H_2O.
(ii) Oxyluciferin′ + luciferase $\rightarrow$ oxyluciferin + luciferase′.
(iii) Luciferase′ $\rightarrow$ luciferase + $h\nu$.

The reaction (i) is catalysed by luciferase, and the product, oxyluciferin, carries a quantum of energy represented by ′. This is transferred to the enzyme itself in reaction (ii), and is liberated in reaction (iii), as a quantum of light energy, $h\nu$.[59] The oxidation of luciferin, like that of haemoglobin, is reversible; the oxyluciferin can be reduced to luciferin again by nascent hydrogen or exposure to light.[105]

The quantity of heat set free in this reaction is exceedingly small. In the case of *Pyrophorus* it is judged to be less than $\frac{1}{80,000}$ of that produced by a candle flame of equivalent brightness[105]: at least 98 per cent. of the energy employed appears in the form of light.[36] In colour the light varies according to the species from greenish blue to reddish golden. Analysed spectroscopically it is found to be entirely free from ultra-violet, and to form a continuous spectrum over a very narrow range of wave lengths (520-650$\mu\mu$ in *Photinus*, 486–720$\mu\mu$

in *Pyrophorus*, 518–656$\mu\mu$ in *Lampyris*) occupying just about that zone to which the human eye at least is most sensitive.[105] It is estimated that some 37–38 *Pyrophorus*, the most luminous of insects, with all their organs functioning, can illuminate a room with about the same intensity as one candle.[36]

Control of light production—Even in the primitive forms, such as Collembola, the production of light is subject to some degree of regulation by the insect. It may be increased by mechanical stimuli (in *Onychiurus armatus* [61]), or take place only as a result of stimulation (in *Achorutes muscorum* [151]). Since oxygen is needed for its production this effect of stimulation is perhaps simply the result of increased oxygenation of the tissues following increased activity. But in the luminescent beetles, the production of light is fully under the control of the insect: in *Lampyris* and *Pyrophorus* the steady glow is maintained only during certain hours of the day; in *Luciola* and *Photinus* the light is produced in rhythmic flashes with a characteristic frequency and duration.[22] Here the control lies in the central nervous system. The rhythmical flashing of *Luciola* ceases after decapitation, but can be induced again if the severed nerve cord is stimulated [165]; and illumination of the head alone, with the remainder of the body in darkness, causes an immediate reflex extinction of the light.[49]

If the light emitted by *Pyrophorus* is analysed with a photo-electric cell it nearly always shows rhythmic changes in intensity at a rate of about 300 per minute at the outset, falling to 150 per minute, and amounting to 5–6 per cent. of the maximal intensity. The records of such changes recall those of an incomplete tetanus in a contracting muscle, and are regarded as due to rhythmic discharges from the nerve centres to the organs.[58] But the mechanism by which the nervous system brings about control is uncertain. Dubois claimed that in Pyrophorids the luminous organs are enclosed by special muscles, which control the entry of blood. When this enters the organ the luciferase in solution in the blood reaches the luciferin in the granules of the photogenic cells and light is produced; when the blood flow is arrested the light becomes dim or is extinguished; and while the light is shining the muscles are said to make rhythmic movements.[36] But in Lampyridae the emission of light may be general throughout the luminous organ or confined to a few discrete areas.[102] There is clearly some form of local control. According to one view this is a nervous regulation of the supply of air to the tracheal endings. Elaborate contractile structures have been described in the tracheal end cells which are credited with controlling mechanically the supply of oxygen to the tracheoles.[31, 49, 148]. Others favour the view that the nerves end in the photogenic cells themselves.[58] It is possible then that the metabolic activity of these cells might result in the extraction of fluid from the tracheal endings (p. 186) and so improve indirectly the oxygen supply.[104, 169]

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Chapter XI

Digestion and Nutrition

ORGANIC MATERIAL of almost every kind in nature may serve as food for insects. The mouth parts in the different groups are adapted for chewing solid substances such as foliage or wood, the bodies of other creatures, or organic refuse of any kind ; for collecting exposed fluids such as the nectar of flowers, honey dew, or exudates from decaying matter ; or for piercing the integument of animals or the tissues of plants and sucking out their juices.[207, 243]

The digestive system is correspondingly modified. In some insects it must cope with an abundant dilute fluid ; in others, almost every trace of water must be conserved. In some it has to digest only a few simple sugars ; in others even the resistant skeletal proteins, such as keratin or collagen, or carbohydrates such as cellulose must be broken down.

The alimentary canal is a tube of epithelium running a straight or convoluted course from the mouth to the anus. In the head it is connected to the body

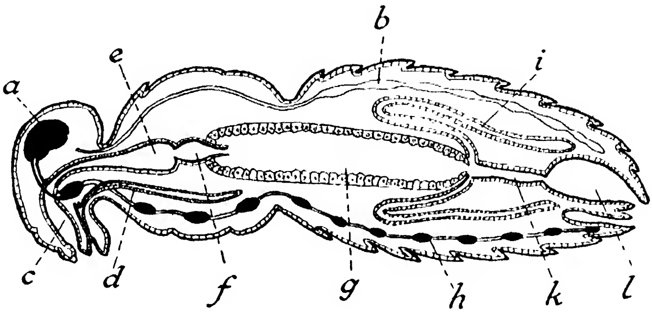


FIG. 214.—Diagram of alimentary system of an insect (*after* SNODGRASS)

a, brain ; *b*, heart ; *c*, pharynx ; *d*, salivary gland ; *e*, crop ; *f*, proventriculus ; *g*, mid-gut; *h*, ventral nerve cord ; *i*, Malpighian tubes ; *k*, small intestine (hind-gut) ; *l*, rectum.

wall by muscles ; elsewhere its coils are supported as a rule only by tracheal branches. It consists of three primary divisions (Fig. 214) : a 'fore-gut' derived from the stomodoeum, lined with cuticle continuous with that covering the surface of the body, a 'hind-gut' derived from the proctodoeum, again with a cuticular lining, and an endo-dermal 'mid-gut' uniting these two. All these parts have a muscular coat innervated from the visceral nervous system ; the fore-gut and the greater part of the mid-gut from the frontal and pharyngeal ganglia, the hind-gut and the posterior region of the mid-gut from the hindmost ganglion of the ventral nerve cord.[125] All segments of the gut show peristaltic and churning movements which serve to mix the contents and carry them along. The digestive enzymes which hydrolyze the food into products capable of

absorption, are secreted partly by the ' salivary glands ' (dermal glands associated with the mouth appendages), but chiefly by the mid-intestine. All segments of the gut may take part in absorption to a varying extent.

THE FORE-GUT

Anatomy and histology—The ' buccal cavity ', forming the commencement of the fore-gut, is followed by the ' pharynx ' with an elaborate musculature concerned with the ingestion and deglutition of the food.[207, 243] Then follows the ' oesophagus ', which may be a simple narrow tube leading to the mid-gut, as in Collembola, Hemiptera, &c., but which is often modified to form a ' crop '. Typically the crop is a symmetrical dilatation of the hind part of the oesophagus, as in the cockroach, in caterpillars, in predaceous Coleoptera, in the bee, &c.; sometimes it forms a lateral dilatation, as in *Gryllotalpa*, certain termites, the larva of the ant lion, and in Curculionidae; sometimes it is a diverticulum separated from the oesophagus by a long and narrow tube, as in adult Diptera, the larvae of Cyclorrhapha, and in most adult Lepidoptera (Fig. 215). The fore-gut is separated from the mid-gut by the ' cardiac sphincter ', and at this point it may be modified to form a muscular ' proventriculus '. The epithelium of the fore-gut is generally continued beyond the cardiac sphincter as a fold, the ' oesophageal invagination ', which projects into the commencement of the mid-gut.

Histologically, the fore-gut is made up of a cuticular coat, smooth, spiculated, or bearing hairs or teeth as the case may be, laid down by a rather thin epidermis resting on a basement membrane. Outside this there is a relatively thick muscular coat with longitudinal muscles inside and circular muscles outside; the whole enclosed in a connective tissue sheath bearing nerves and tracheae.

Function of the crop—The crop may simply form a temporary reservoir for food. In caterpillars the food is merely stored and passed on from time to time as the mid-gut is evacuated. In the tsetse-fly *Glossina*, great quantities of blood are taken to the crop and transferred to the mid-gut as required.[130] The same is true of *Musca*, *Calliphora* and other flies that feed on exposed fluids of all kinds.[89] In *Haematopota* the blood goes first into the stomach, but when this is full it is diverted to the crop [39]; and in mosquitos, whereas blood is always passed into the stomach, and appears only in traces in the diverticula of the oesophagus, sweet juices or exudates from fruit go first into the crop, and are then conveyed at intervals to the mid-gut.[24, 139]

But in some insects the food in the crop is mixed with the digestive juices. In Orthoptera the secretion of the salivary glands is swallowed with the food, the secretion from the mid-gut is passed forward, and the crop is probably the chief region where digestion takes place.[174, 251] It is the main seat of digestion also in Carabids and other beetles [174, 195]; and probably in the ant lions and their allies. The food of blow-fly maggots contains the enzymes present in their own excreta (p. 277) and these must continue to act while the food is in the crop. In the bee, the nectar of flowers is mixed with enzymes from the salivary glands and is converted into honey in the crop or ' honey stomach '.[193]

On the other hand, the crop is of little importance in absorption. Aqueous solutions can be exposed in the crop dissected from the mosquito for several

days without drying up.[24] The crop of the cockroach used as an osmometer is practically impermeable to water,[66] and no sugar will pass through it.[1] Olive oil, however, will pass through, and in the living cockroach fat may be absorbed in it; for if the crop is ligated and the insect fed with fat, this will appear only in the cells in front of the ligature,[1] or in cells overlying localized fat.[192]

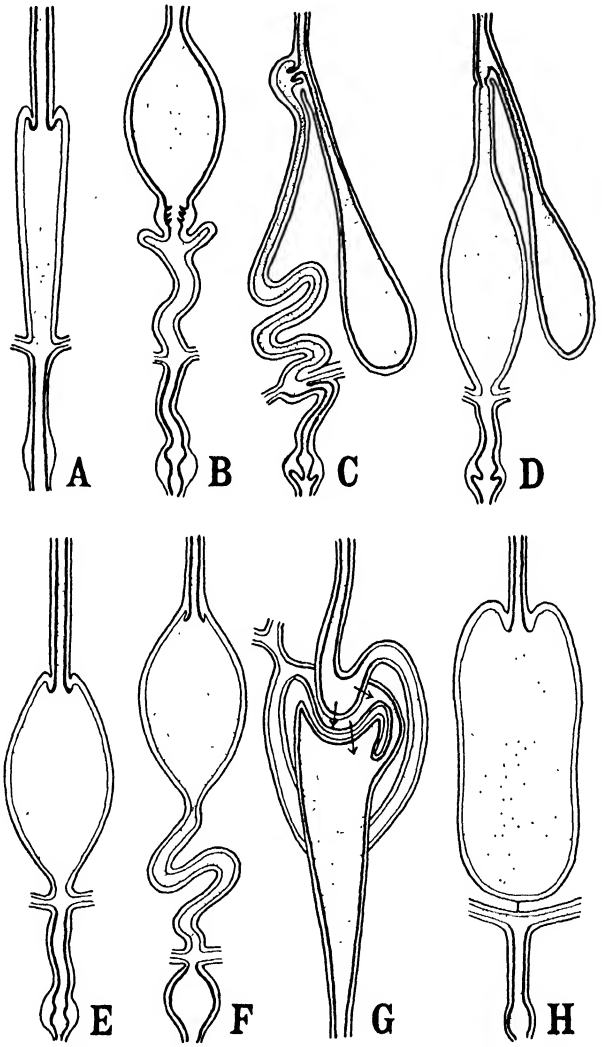


FIG. 215.—Diagrams showing some of the modifications of the alimentary system

Fore-gut and hind-gut indicated by a heavy line internally. *A*, in primitive insects and many larvae; *B*, in Orthoptera, Odonata, Hymenoptera, many Coleoptera; *C*, in higher Diptera; *D*, in Diptera Nematocera, Lepidoptera; *E*, in Siphonaptera, Siphunculata; *F*, in many Hemiptera Heteroptera; *G*, in Coccidae; *H*, in larvae of Hymenoptera Apocrita and in larvae of *Myrmeleon* and other Neuroptera.

Diverticula from the oesophagus may have some accessory functions. In the larva of the saw-fly *Lophyrus*, a pair of small pouches serve as receptacles for the useless resin from the surface of the pine needles on which the larvae feed.[190] In mosquitos they seem to take up any air swallowed with the food.[139] In many insects the crop is the chief receptacle for the air that is swallowed and

distends the body at ecdysis (p. 28); in those Lepidoptera which take no food in the adult state it retains this as its sole function.[214]

Function of the proventriculus—The proventriculus shows all degrees of development, from a simple sphincter lined with soft cuticle, to a powerful muscular organ armed with spines and teeth. It occludes the passage from the fore-gut to the mid-gut. In many beetles it probably transmits the contents of the crop in small quantities at a time, and perhaps acts as a sieve.[224] But in some insects it undoubtedly serves to crush and triturate the food.[63] In the Cerambycid larva *Xystrocera globosa* no proventriculus is present, and the particles of wood in the mid-gut are relatively large; whereas in *Macrotoma palmata*, which has a well developed proventriculus, the contents of the gut are finely pulverized.[144]

This gizzard-like function has been most clearly demonstrated in the cockroach. Here the proventriculus bears in front six powerful radial teeth invested by a strong compressor muscle; behind these are two corresponding rows of hairy cushions (Fig. 216). The hind part is just a sphincter, the fore part is certainly a crushing apparatus. For the solid particles are always smaller in the mid-gut than in the crop; and the old lining of the proventriculus, which is cast off at moulting, is crushed and broken up and passed in small fragments in the faeces, as soon as the new proventriculus has hardened. After crushing, the products are first returned to the crop, exposed to the digestive juices, and then admitted to the mid-intestine.[67]

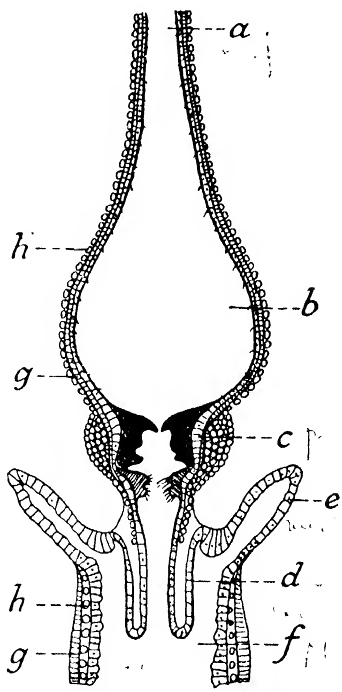


FIG. 216.—Fore-gut of cockroach *Blatta orientalis* (*after* SNODGRASS)

a, oesophagus; *b*, crop; *c*, proventriculus with large teeth in front and hairy cushions behind; *d*, oesophageal invagination; *e*, cacca of mid-gut; *f*, mid-gut; *g*, longitudinal muscles; *h*, circular muscles.

In the flea, the proventriculus is lined by long curved backwardly directed spines. No crop is present, and during digestion the proventriculus contracts rhythmically, driving the spines backwards into the mid-gut and probably breaking up the corpuscles in the ingested blood.[72]

In the bee the proventriculus is called the 'honey stopper'. Here, and in other Hymenoptera, it forms an elongated plug with an elaborate internal musculature, which projects far into the crop, its lumen occluded by four converging lips with short curved spines. In spite of its strong muscles, this organ seems incapable of crushing grains of pollen.[245] But it will seize these grains and transfer them into the stomach without removing fluid with them. If the bee is fed with syrup containing a suspension of pollen grains, the crop remains turgid with fluid long after all the grains have been removed.[249]

The oesophageal invagination, which follows the proventriculus or cardiac sphincter, is often referred to as the 'oesophageal valve', in the belief that it forms a mechanism for preventing regurgitation from the mid-gut to

the fore-gut. It may have this function in the bee,[5, 249] but in most insects such reflex is prevented by the cardiac sphincter,[204] which, indeed, in many forms extends into the invagination.[253] In most insects the invagination is quite unsuited by its structure to act as a valve ; having too rigid walls, or being so disposed that the mid-gut contents cannot exert pressure on its outer face.[252, 253] We shall discuss its function in connexion with the peritrophic membrane (p. 260).

THE PERITROPHIC MEMBRANE

Peritrophic membrane : type i—The epithelial cells of the mid-gut are devoid of cuticle, but in most insects they are protected from the contents of the gut by a delicate detached sheath, the 'peritrophic membrane'. There are two types of peritrophic membrane, formed in two different ways. In the first type, which occurs in Phasmids,[41] Acridiids,[54] Ephemeroptera,[56] Odonata,[239] caterpillars,[223, 236] *Tenebrio*, *Hydrophilus* and other beetles,[56, 236] and *Apis*,[166] *Vespa* [90] and their larvae,[182] the membrane is made up of concentric lamellae, independent or loosely attached to one another. It is produced by the separation of thin sheets from the surface of the cells throughout the length of the mid-gut. The mid-gut cells generally bear a striated border (p. 262), and in these insects each new sheet appears as a limiting membrane at the surface of this border (in the bee,[232] in *Galleria* larva [223]) which is raised and detached by the pouring out of secretion below it (Fig. 217). When the newly formed lamellae in the bee are stained, they often show polygonal areas corresponding to the cell surfaces by which they were laid down.[56] As in the formation of the body cuticle, this process is sometimes regarded as a secretion of substance which condenses to a membrane,[236] sometimes as the transformation of a part of the cell surface ; but there is no real distinction between these alternatives. Sometimes the lamellae carry with them a part of the striation from the cells (in *Aeschna*,[239] in *Apis* [166, 249]), but they cannot be regarded merely as detached striated borders, because the lamellae contain chitin while the striated border does not.[56]

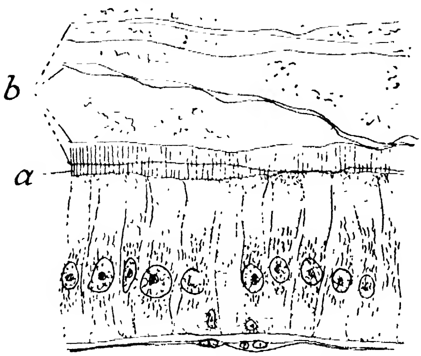


FIG. 217.—Section through mid-gut of nymph of *Cloëon* sp., showing origin of peritrophic membrane of type i (*after* v. DEHN)

a, striated border ; *b*, successive laminae of peritrophic membrane shed off from the mid-gut cells.

In the larvae of the wasp and bee, at least half a dozen membranes of this kind are set free each day [182] ; and since in these larvae the mid-gut forms a closed sac, the contents of the gut come to be invested in a great number of superimposed envelopes. In the larva of *Aeschna*, even in the absence of feeding, about two such peritrophic sacs may be discharged each day.[10]

Peritrophic membrane : type ii—In the second type, which occurs in the larvae and adults of Diptera,[253] in the earwig [51, 253] and perhaps in termites,[152, 253] the peritrophic membrane consists of a single uniform layer. It

is secreted in viscous form by a group of cells at the anterior limit of the mid-gut (Fig. 218), passes through an annular cleft between the oesophageal invagination and the mid-gut, and in so doing solidifies to form a homogeneous tube.

An annular mould of this type was first recognized in larvae of *Ptychoptera* [82] and *Chironomus* [236]; it has since been described in the larvae of mosquitos (Fig. 219, A) and many other Nematocera,[253] in the larva of *Eristalis* [10] and in adults of *Glossina* (Fig. 219, B),[252] *Calliphora* [89] and other flies. In these insects the mid-gut forms the outer wall of the mould, its constrictor muscles being generally exaggerated at this level and so disposed as to provide the pressure

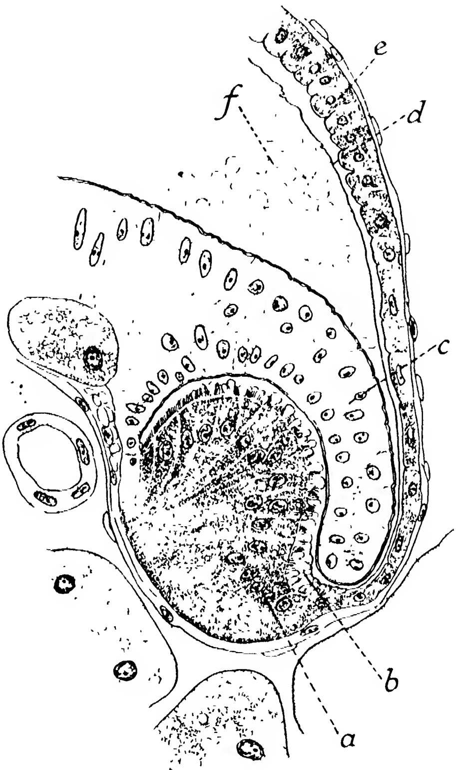


FIG. 218.—Formation of peritrophic membrane (type ii) in *Glossina* (*after* WIGGLESWORTH) (*cf.* Fig. 219)

a, cells secreting substance of membrane; **b**, vesicles of secretion; **c**, invagination of fore-gut, forming press; **d**, peritrophic membrane; **e**, mid-gut epithelium; **f**, freshly ingested blood in lumen of gut.

necessary for moulding; this is well seen in the earwig (Fig. 219, C),[253] in *Calliphora* adult [89] and in the larva of the Syrphid *Syritta*.[122] The inner wall of the mould is provided by the oesophageal invagination, which may be composed of large tense cells forming a solid plug (as in the larvae of *Calliphora*,[168] *Eristalis* [10] and *Syritta* [122] and the adult of *Glossina* [252]), or the cells upon the outer face of the invagination may lay down a ring of thick and rigid cuticle (as in *Forficula*,[253] *Anopheles*,[253] *Rhyphus* [253] and several other Nematocera [194, 216]). Longitudinal muscles may be inserted into this ring and serve to draw it forwards [253]; and between the walls of the invagination are often blood sinuses, which can be distended and so force it back again.[10] On the

outer wall of the invagination there are often rows of spines (in *Mycetophila*,[106, 140] *Simulium*,[216] and *Tabanus*[209] larvae) and then the backward and forward movements will serve to draw out the peritrophic membrane. In other cases, such as the Muscoid flies, the membrane is probably drawn backwards

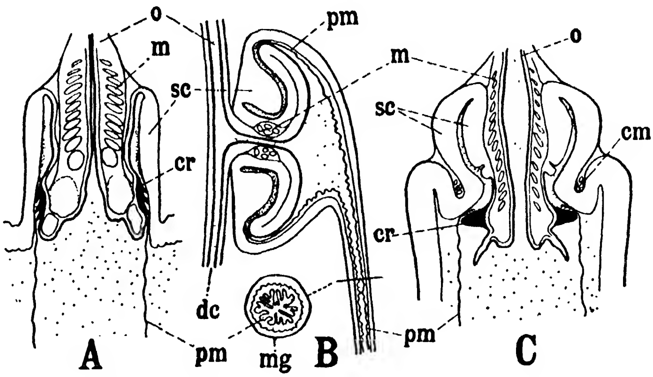


FIG. 219.—Annular moulds producing peritrophic membrane (type ii)

A, larva of mosquito, *Anopheles*; *B*, tsetse fly, *Glossina*; *C*, earwig, *Forficula*. (*After* WIGGLESWORTH.) *cr*, cuticular ring forming inner wall of press; *cm*, circular muscle compressing outer wall against this ring; *dc*, duct of crop; *m*, sphincter muscle; *mg*, mid-gut in cross-section; *o*, oesophagus; *pm*, peritrophic membrane; *sc*, cells secreting the substance of the membrane.

by the peristaltic movements of the gut.[89] In the unfed larva of *Eristalis* the membrane is continuously secreted at the rate of about 6 mm. an hour.[10]

The peritrophic membrane of this second type is always of uniform circumference throughout its length. Where there are diverticula from the mid-gut, as in Muscid or Culicid larvae, it bridges their openings without entering them. Where the gut is wider than the mould in which the tube is formed, the mem-

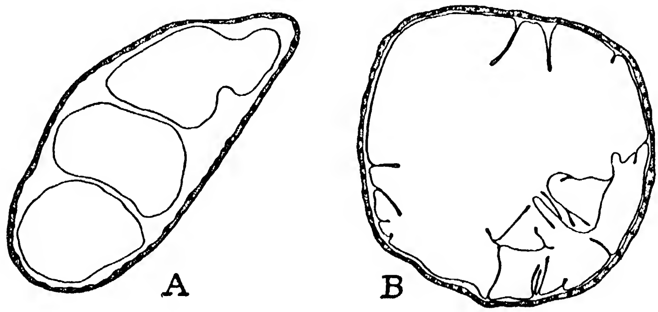


FIG. 220

A, transverse section of dilated segment of mid-gut in larva of *Musca*. Peritrophic membrane is thrown into coils and is then cut through in several places (*cf.* Fig. 226). *B*, transverse section of mid-gut of *Glossina* adult. The lumen is smaller than the peritrophic membrane, which is thrown into longitudinal folds. (*After* WIGGLESWORTH.)

brane may form loose coils within the lumen, as in *Tabanus*[209] and *Lucilia*[102] larvae (Fig. 220 A, 226); while when the gut is narrower than the mould, as in many parts of the intestine in *Glossina* adults,[252] the membrane is thrown into longitudinal folds (Fig. 220, B).

The oesophageal invagination in most insects is a thin-walled structure. In caterpillars it is often cleft along one side, as in *Vanessa*[97] or split up into

two or three lobes as in *Galleria*,[148] *Chimabache* (Fig. 221), *Ephestia*, &c.[253] When food passes through, these lobes are pressed against the wall of the mid-

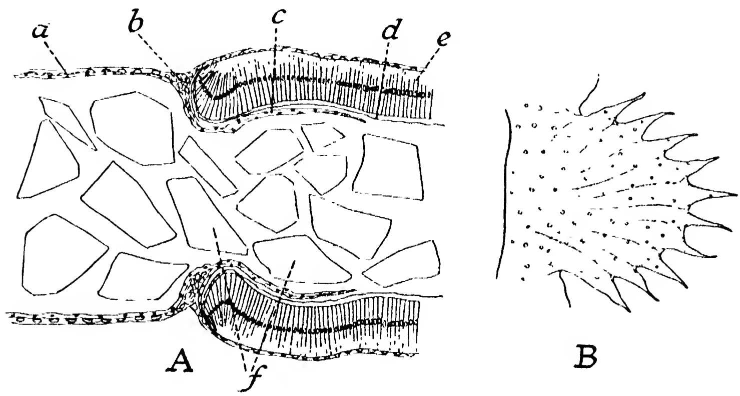


FIG. 221

A, longitudinal section of junction of fore-gut and mid-gut in the caterpillar of *Chimabache*; B, one of the three leaflets of the oesophageal invagination seen in surface view. (*After* WIGGLESWORTH.) a, fore-gut; b, circular muscle; c, oesophageal invagination; d, peritrophic membrane; e, mid-gut; f, fragments of foliage in lumen of gut.

gut, and thus ensure that the contents enter within the peritrophic membrane. That is perhaps the primary function of the invagination. But where the peritrophic membrane is covered by the invagination, it is compressed against the surface of the gut; in this region it is more compact and less obviously laminated; and at the anterior limit of the mid-gut it generally remains attached to the cells. It may thereby give a false impression of taking origin solely from these cells (as in *Aeschna*,[239] *Chrysopa*,[146] the larva of the bee,[155] *Xylocopa* [25] and in caterpillars [27]).

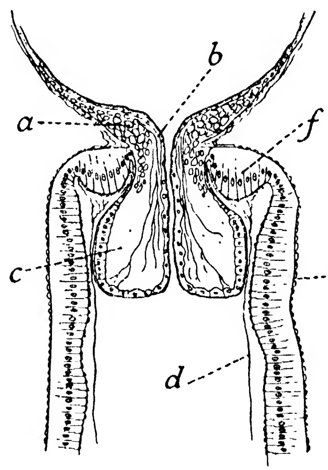


FIG. 222.—Oesophageal invagination of larva of saw-fly (Tenthredinidae) (*after* WIGGLESWORTH)

a, circular sphincter muscle between fore-gut and mid-gut; b, longitudinal muscle running into oesophageal invagination; c, blood sinus distending the invagination; d, peritrophic membrane; e, circular muscle of mid-gut; f, ring of cells with peritrophic membrane adherent to them.

In other insects, such as saw-fly larvae,[253] the adults of Neuroptera [146] and Hymenoptera,[253] the thin-walled invagination can be distended with blood, and in this way also may exert pressure upon the wall of the mid-gut (Fig. 222). In such cases, even though it be admitted that the greater part of the membrane is formed by delamination throughout the mid-intestine, it is possible that some may be provided by the cells beneath this simple press. It has in fact been suggested that in *Polistes*,[169] *Bombus*, *Apis* and other insects [253] the peritrophic membrane may have a double origin, combining the features of the two types described above, and perhaps representing a stage in the evolution of the second type from the first.

Composition and function of the peritrophic membrane—The peritrophic membrane has the same components as the inner layers of the cuticle: a basis of chitin, with protein incorporated in it.[88, 252, 253] This applies both to the laminae separated from the surface of the mid-gut, and to the membranes secreted by the cardiac cells.[56]

It is generally regarded as protecting the mid-gut cells from abrasions by hard fragments in the food, replacing in this respect the mucous involucrum which lubricates the gut of vertebrates.[15, 152, 253] This belief is supported by the fact that the membrane is absent in Hemiptera and in adult Lepidoptera, which feed only upon fluids, and in many of the blood-sucking insects such as Tabanids, mosquitos, fleas and lice. It is absent in many carnivorous Coleoptera (Carabids and Dytiscids) in which the mid-gut cells break down completely during secretion (p. 264). And it is absent in *Gryllotalpa*, in which the mid-gut is very short and is protected by four delicate chitinous lamellae, attached to the hind margin of the oesophageal invagination, which reach as far back as the commencement of the hind-gut.[51] But it is curiously missing also in the ant,[56] and in the larva of *Anthrenus* (Dermestidae),[151] in which its protective function might seem to be required.

The permeability of the membrane varies somewhat from one insect to another; it has been tested with different dyes in *Dixippus*,[41] termites,[152] *Apis*, *Vanessa* larva, and *Calliphora*.[56] As a rule only dyes with large colloidal particles such as congo red or Berlin blue are arrested by it. To that extent it serves as an ultra-filter. But it offers no hindrance to digestion; for digestive enzymes and the products of their action readily pass through it.[56, 252]

THE MID-GUT

Histology—The mid-gut is made up of cubical or columnar cells, usually much thicker than the epidermis of the fore-gut, a basement membrane, an inner circular muscle coat, and an outer longitudinal. As a rule, the lattice work of muscle fibres covers but a part of the epithelial cells; in many places these are separated from the circulating blood only by the basement membrane and a delicate connective tissue sheath.

Internally the cells are generally bounded by a striated border, though this may be absent. As in the Malpighian tubes (p. 309) two types of border probably occur [158]; one made up of rod-like elements organically held together to form a rigid structure (the 'honey-comb border'), the other composed of independent hair-like filaments which, though not mobile, can be moved passively about (the 'brush border'). The former is more frequent; the latter is best seen in caterpillars and in saw-flies: in *Cimbex* (Tenthredinidae) the hair-like filaments are 45μ in length, longer than the cells which carry them.[81] In the cardiac end of the mid-gut of *Phlebotomus* (Dipt.) the independence of the rods is demonstrated by the fact that intestinal flagellates multiply among them [4]; in *Ptychoptera* larvae they are often inclined at an angle to one another.[82]

Goblet cells—Among the cylindrical epithelial cells there may occur a smaller number of 'goblet cells', in which the cytoplasm is reduced, and the cell surface with its striated border is invaginated to form a deep cavity.[97] These cells occur in Ephemeroptera, Plecoptera, &c., but they are most conspicuous in the larvae of Lepidoptera, becoming more numerous towards the

hind end of the gut (Fig. 223).[201] No satisfactory function has been ascribed to them. By some authors they are regarded merely as a resting or senescent stage of the cylindrical cells [55, 201]; in *Pyrausta* larvae transitional forms from one type to the other are described.[31] Others consider them as quite distinct, arising independently from the embryonic cells; in *Galleria* [222] and *Vanessa* [97] larvae no intermediate forms can be detected. Both types are present in the newly hatched larva,[97] or even during the last few days of embryonic life [222]; but this does not exclude the possibility of their being exhausted cells, for even at this stage digestion of the yolk and of the egg-shell has begun.[201]

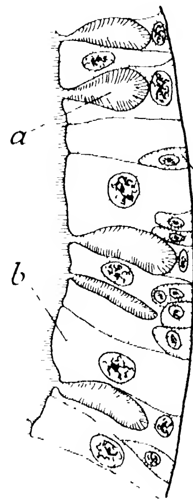


FIG. 223.—Epithelium of mid-gut in larva of *Vanessa urticae* (*after* HENSON)

a, goblet cell; b, columnar cell; c, interstitial or regenerative cells.

Secretory changes—There is no doubt that secretion in the mid-gut can take place without the cells showing any alteration, either in the living state (in *Chironomus* [235] and mosquito larvae [259]), or in fixed preparations (in *Vespa*,[90] in caterpillars and other insects [97, 157]). But many kinds of visible changes may occur. The nucleus is sometimes described as giving out deeply staining granules or chromidia from its inner pole, which migrate towards the lumen and give rise to vacuoles (in the saw-fly *Nematus*,[107] in *Acridium*,[105] in *Musca* larva [31]). In the beetle larva *Rhagium* [68] and in the cockroach [91] the nucleus shows no change, but the mitochondria in the basal region of the cells are believed to be associated with the separation of secretory material, which then, under the influence of the Golgi apparatus, appears in the form of granules in the region of the nucleus. The granules become vacuoles, which may remain separate or fuse into a single cavity. The contents of the separate vacuoles may escape as little vesicles between the rods of the striated border; or the apex of the cell may become so distended that the striated border disappears, and a single large globule is set free (in *Ptychoptera*,[82] *Pyrausta*,[31] *Chrysopa*,[146] *Galerucella* [177]). Or the vacuolated tips of the cells may be nipped off and break down in the lumen (in *Tabanus*,[49] *Glossina*,[252] *Calliphora*,[89] *Aphis*,[240] and other Hemiptera [175]). Or little spheres of cytoplasm may be extruded through the striated border (in *Deilephila*,[55] *Bombyx rubi*,[96] and other Lepidoptera [214]).

These changes have been observed sometimes in fresh material [82, 96]; but to what extent they are a part of normal physiology has been a subject of much controversy. It is certain that vesicular secretion of this kind can be produced artificially. In the living larva of *Chironomus* no vesicles occur; but they appear at once if the larva is dissected or compressed.[235] In the bee [166, 171] and in other insects [213] they can be seen forming during fixation. In the mid-gut caeca of mosquito larvae they are absent during life,[259] and in sections they appear only after certain fixatives.[24] But it is possible that these technical procedures cause the cell contents to protrude in a manner resembling the normal secretory changes.[213] In the larva of *Vanessa* [97] the 'secretion globules' are thought to result from disintegration of the cells, and not to represent a secretory process; they occur only in very large columnar cells, and are not

seen earlier than the end of the 2nd instar. These epithelial changes may appear indiscriminately throughout the gut. But sometimes, as in *Deilephila* [55] and in *Aphis*,[240] waves of secretory activity seem to pass from before backwards when a meal is taken; vacuolation, distension, and rupture of the cells apparently succeeding one another in an orderly manner.

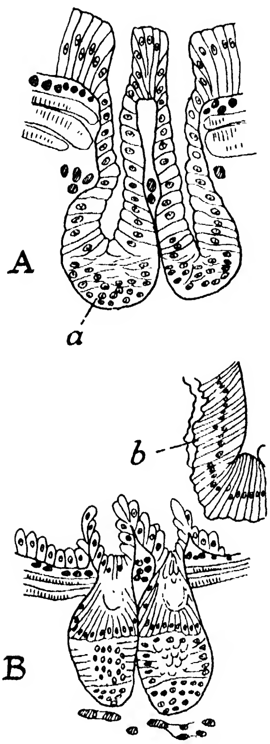


FIG. 224

A, mid-gut epithelium in *Hydrophilus* during resting stage; showing two regenerative crypts (*a*). *B*, the same with the old epithelium (*b*) cast off, and new epithelium being regenerated. (*After* RENGEL.)

In the types of secretion so far described, the nucleus remains intact and the cells can recover; this is termed 'merocrine secretion'. But in some insects a variable number of the cells break down completely; this is called 'holocrine secretion'. There is no sharp distinction between the two types; in the bee, both occur together [245]; in Orthoptera, secretion is merocrine during continuous small meals, holocrine when a meal follows a period of fasting.[201] In *Tabanus*[49] and *Dytiscus*,[20] vacuoles alone may be discharged; or the inner border of the cell may give way and a mass of granular cytoplasm, often carrying the nucleus with it, be set free into the lumen. In the flea,[72] and in *Galerucella* (Col.),[177] while some cells show little change, others break away entire and disintegrate in the cavity of the gut. In *Hydrophilus* (Fig. 224),[81] *Tenebrio*,[17] and other beetles,[157] the entire lining of the mid-gut is shed off and replaced every forty-eight hours.

Regeneration—Among the active epithelial cells there are small basal, embryonic or replacement cells (Fig. 225). These may be scattered singly along the gut as in caterpillars and in Diptera; or they may be collected at intervals in small groups or nidi, as in Orthoptera, *Odonata*, *Plecoptera*, &c.; or they may form large crypts projecting through the muscular coat and standing out like tags or villi all over the outer surface of the gut, as in Carabids and many

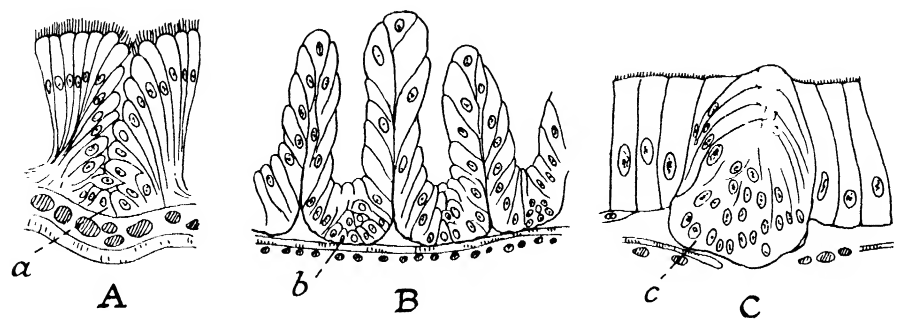


FIG. 225.—Regenerative cells of the mid-gut epithelium

A, *Stenopelmatus* (Orthopt.) (*after* DAVIS); *B*, *Apis* (*after* SNODGRASS); *C*, *Dytiscus* (*after* RUNGIUS). *a*, nidus of regenerative cells; *b*, cells in the folds of the gut; they are probably both secretory and regenerative; *c*, regenerative crypt.

other Coleoptera.[201] In *Dytiscus* these three arrangements may appear at successive stages in development.[20] As the epithelial cells degenerate, these basal cells grow to replace them.[81, 105] This may be a gradual process, as in *Galleria*,[222] *Vanessa*,[97] or *Popillia*,[138] where all through larval life they are actively dividing to provide for renewal of the epithelium ; or it may occur rhythmically, as in those Coleoptera showing holocrine secretion, and sometimes (perhaps in association with moulting) in the cockroach.[91]

In some insects the epithelium of the mid-gut appears to be renewed completely at each moult, as in Thysanura and Collembola, *Galleria*,[222] and *Psychoda*,[94] termites,[247] *Dermestes* and *Anthrenus* [151] ; in others complete replacement takes place only at pupation, the larval epithelium being generally shed into the lumen, where it breaks down to form an amorphous mass known as the 'yellow body', as in *Vanessa*,[97] *Popillia*,[138] *Ptinus*, &c.[142] The imaginal epithelium may be formed from the replacement cells of the mid-gut, or from groups of interstitial cells at the points of union with the fore-gut and hind-gut. In *Calliphora* the mid-gut is broken down completely at pupation and its contents liberated in the body cavity.[168]

Absorption—There is no phagocytosis of food particles by the mid-gut cells of insects ; all the products of digestion are absorbed in solution. In most insects there is no doubt that secretion and absorption are carried out by the same cells. Sometimes the cells are thought to go through alternate phases of secretion and absorption, as in *Deilephila* [55] and the cockroach [91] ; but there is no clear proof of this. The distribution of absorbing cells has been tested by feeding insects with iron salts and with dyes ; but these methods have given contradictory results on the same insect, notably the cockroach, in the hands of different authors.[51, 91, 213] The histological stages in absorption have been described (in *Chrysopa*,[146] *Musca* larva,[31] &c.) but the conclusions rest on a more or less arbitrary seriation of the appearances seen in sections. Fat is absorbed in the mid-gut [51, 213] ; in *Galleria* it is taken up by the columnar cells, never by the goblet cells [222] ; and there is evidence that it is first hydrolyzed ; for if *Tenebrio* is fed with fat dyed red, the fat appearing in the cells is colourless.[17]

Vacuolated mid-gut cells are generally thought to be engaged in secretion ; but it is probable that the vacuoles may sometimes represent absorbed material. Thus in *Notonecta* the dilated anterior part of the mid-gut is regarded as the chief seat of secretion on account of the great vacuolation of the cells [23] ; but in other bugs (*Cimex* and *Rhodnius* [257]), it is known that this region is concerned only in absorption of the fluid from the meal.

Cell inclusions—Globules of fat are often present in the cells of the mid-gut. In many cases this is probably storage fat ; for in the cockroach it appears after feeding with sugar,[213] and in the mid-gut of blow-fly larvae it is present after feeding on meat.[102] Crystals, apparently composed of protein, occur within the nuclei in larvae of Lamellicorn beetles [149] and *Tenebrio*,[17, 80] but their significance is not known. Granules of many kinds are often present in the cells, and in the mid-gut of the bee and some other insects these inclusions are composed of lime.[117]

Functional subdivisions of the mid-gut—In most insects the structure of the mid-gut is more or less uniform throughout ; though there may be one or more groups of diverticula or caeca which serve to increase its secretory and

absorptive area, as in Orthoptera, Lamellicornia and many Dipterous larvae. In some, however, there is a clear division into regions. We have already discussed the large deeply staining columnar cells in the cardia of Diptera, which secrete the peritrophic membrane (p. 259). In most Nematocerous larvae, such as *Ptychoptera*,[82] *Anopheles*,[79] *Culex*,[101] this zone is followed by a group of caeca in which both secretion and absorption are believed to take place. The remainder of the mid-gut is a long straight tube composed of two segments: an anterior in which the cells are clear and free from granules, regarded on histological grounds as absorbing cells, and a posterior in which the cells, regarded as secreting cells, are opaque white when fresh, and filled with deeply staining granules.

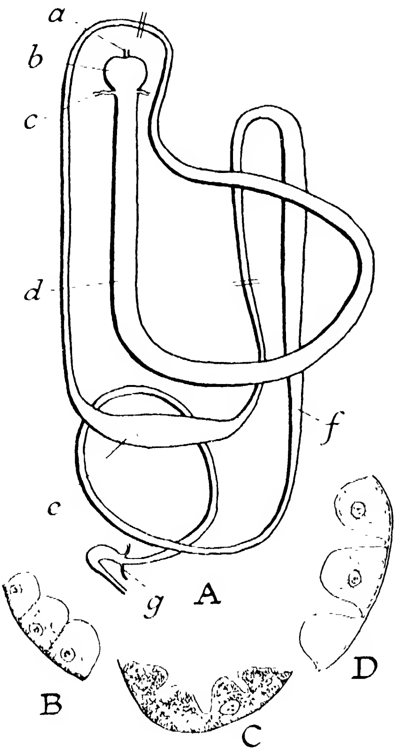


FIG. 226

A, mid-gut of *Lucilia* larva showing division into three segments (points of separation indicated by double lines). B, C, and D, detail of epithelium in the anterior, middle and posterior segments respectively. (*After* HOBSON.) a, oesophagus; b, proventriculus; c, mid-gut caeca; d, anterior segment of mid-gut; e, middle segment (dilated at this point, with peritrophic membrane thrown into coils); f, posterior segment; g, Malpighian tubules.

In *Lucilia* larvae the long coiled gut consists of three segments: the anterior and posterior, which are similar histologically and consist of vacuolated cells, secrete digestive enzymes; the short middle zone is strongly acid (p. 274) but secretes no enzymes (Fig. 226). In these larvae the food is introduced in a fluid state from the crop into the mid-gut. Here it is mixed with digestive enzymes; and water and simple products are absorbed. By the time it reaches the posterior segment it has been concentrated to a pasty consistency; it dissolves in the alkaline fluid of that zone and is digested and absorbed.[102]

In the adult tsetse-fly *Glossina*, there is a still more obvious division of labour in the mid-gut. The gut is long and coiled (Fig. 227). In the anterior half the cells are small and pale staining, and in this segment the blood is thickened to a friable consistency by the absorption of water; but no enzymes are present and no digestion takes place. This region gives way abruptly to a middle segment of large deeply staining cells which secrete digestive enzymes; the blood is blackened when it comes in contact with this epithelium. This segment gradually changes to a narrow posterior segment which is probably absorptive.[252] A similar though less striking subdivision occurs in *Calliphora* and other Muscid flies.

In *Belostoma*, *Ranatra*,[135] *Notonecta* [23] and other Hemiptera-Heteroptera, the mid-gut consists of a dilated stomach separated by a sphincter from a long intestine (Fig. 228). The stomach is often regarded as the chief secretory part because of the vacuolation of its cells,[23, 48] but in the blood-sucking forms *Cimex* and *Rhodnius* it serves merely as a crop; the blood may be retained in

it for several weeks, the excess fluid is absorbed, but the blood is not blackened or digested until it is transferred to the intestine.[257]

Filter chamber.—A characteristic arrangement of the mid-gut exists in Homoptera, in association it is supposed with their habit of feeding on the copious watery juices of plants. The terminal region of the mid-gut comes into intimate relation with the lower end of the oesophagus or the commencement of the mid-gut.[241] In Membracids the end of the mid-gut simply forms a

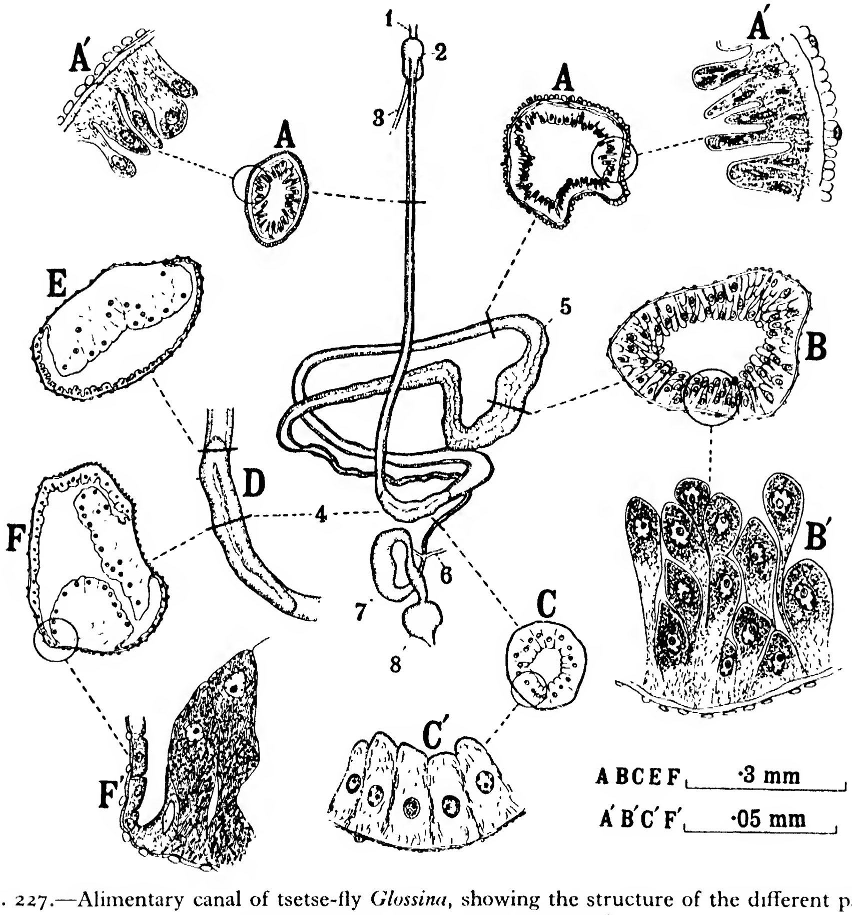


FIG. 227.—Alimentary canal of tsetse-fly *Glossina*, showing the structure of the different parts of the mid-gut (*after* WIGGLESWORTH)

A,A', transverse section of anterior segment ; *B,B'*, ditto of middle segment ; *C,C'*, ditto of posterior segment ; *D*, detail of zone of giant cells containing symbionts ; *E,F,F'*, transverse sections through this zone showing the rod-like organisms within the cells. **1**, oesophagus ; **2**, proventriculus ; **3**, duct of crop ; **4**, giant-cell zone ; **5**, junction of anterior segment with middle segment ; **6**, Malpighian tubes ; **7**, hind-gut ; **8**, rectum.

loop which penetrates the outer wall and applies itself to the epithelial layer of the fore-gut. In Psyllids the hind-gut and oesophagus are spirally wound round one another. In Aleurodids a still more intimate relation exists.[244] In Cercopids the first part of the mid-gut forms a dilated pouch, and the lower end of the mid-gut and a part of the Malpighian tubes lie coiled between its epithelium and connective tissue coat (Fig. 229).[131]

In Coccids the arrangement is more complex, for the associated coils of

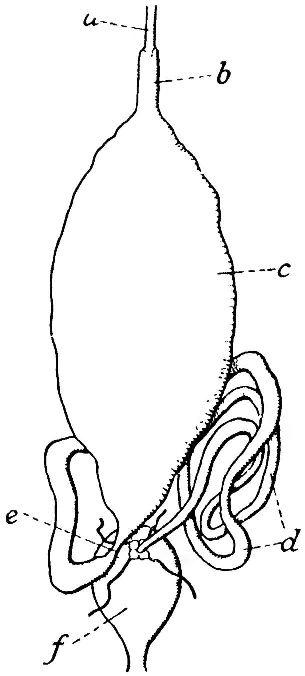


FIG. 228.—Divisions of the mid-gut in *Rhodnius* (*after* WIGGLESWORTH)

a, oesophagus; **b**, narrow 1st segment of mid-gut (*cf.* Fig. 236); **c**, dilated 2nd segment of mid-gut; **d**, narrow coiled 3rd segment of mid-gut; **e**, sphincter between 2nd and 3rd segments; **f**, rectum.

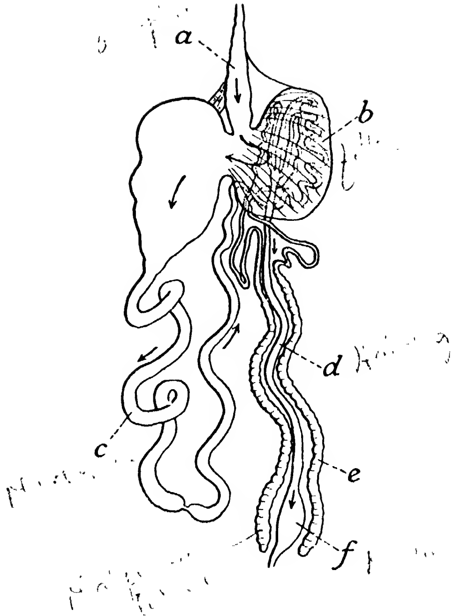


FIG. 229.—Gut of *Thomaspis saccharina* (Cercopidae) (*after* KERSHAW)

a, oesophagus; **b**, filter chamber consisting of coiled endings of mid-gut and Malpighian tubes invaginated into a diverticulum of the oesophagus; **c**, mid-gut; **d**, hind-gut; **e**, Malpighian tubes (two only of the four are shown); **f**, rectum.

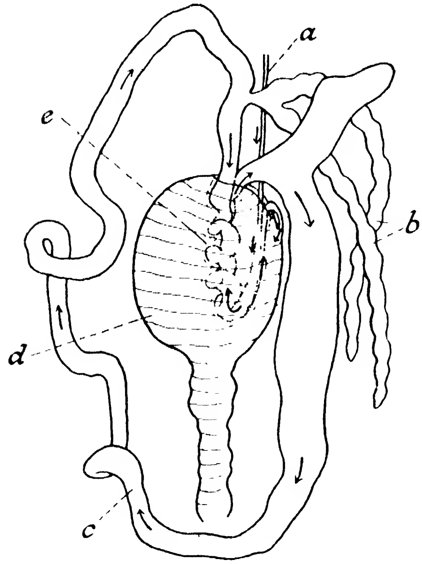


FIG. 230.—Gut of *Lecanium* (Coccidae); showing filter chamber consisting of the first and last segments of the mid-gut closely coiled together and invaginated into the rectum (*after* PESSON)

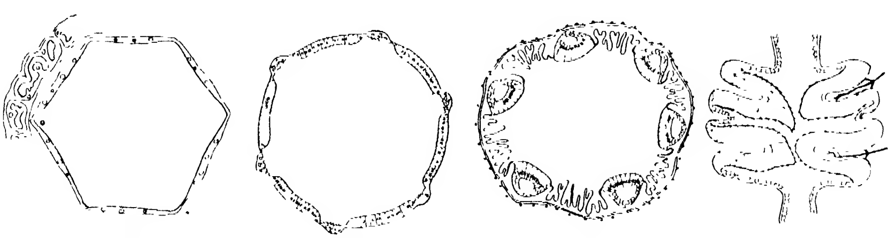
a, oesophagus; **b**, Malpighian tubes; **c**, intermediate segment of mid-gut; **d**, rectum; **e**, filter chamber.

mid-gut are enveloped in the hind-gut. This is well seen in *Pseudococcus*, where the initial and terminal parts of the mid-gut form two loops invested in a connective tissue sheath ; these lie in an invagination of the surface of the rectum ; the intermediate segment of the mid-gut, with a very different histological structure, being free in the body cavity.[170] In *Lecanium* the first and last parts of the mid-gut form a closely interwoven coil like a glomerulus within the invagination of the rectal wall (Fig. 230).[170]

It is believed that these arrangements have a common purpose : they enable the excess fluid in the food to pass directly from the first part to the last part of the mid-intestine. The main digestive segment of the gut is thereby short-circuited, and receives only the valuable constituents of the plant juices.[15, 131] This transference of fluid cannot of course be brought about by simple physical 'filtration' ; the different epithelia must play an active part, as in any other secretory process.[170] Where the related coils are invaginated in the rectum, as in *Pseudococcus* and *Lecanium*, it is possible that a further passage of fluid may take place through the walls of this invagination into the lumen of the rectum [15] ; but since the mid-gut is continuous with the hind-gut this is not a necessity.[170] Perhaps the invagination merely serves to isolate the 'filter chamber' from the general body cavity.

THE HIND-GUT

Histology—The hind-gut is made up of the same layers as the fore-gut, but the epithelial cells are usually larger and they often show a conspicuous vertical striation of the inner border. The cuticle is thinner and, unlike that of the fore-gut, it is readily permeable to water.[1, 66, 88]



A B C D

FIG. 231.—Sections through the rectum of some insects to show the types of rectal glands (*modified after various authors*)

A, *Tenebrio*, showing a part of the investing sheath carrying the Malpighian tubes ; *B*, *Pterostichus* (Carabidae) ; *C*, *Apis* ; *D*, *Calliphora* (longitudinal section).

Towards the hind end the epithelial cells tend to increase in size and to be arranged in six longitudinal folds, as in Thysanura. This wider part is termed the 'rectum,' in distinction from the narrower 'ileum' which precedes it. Smaller cells may lie between the longitudinal folds, as in *Tenebrio* (Fig. 231, A) ; or the large columnar cells may be collected into six cushions radially disposed and occupying a relatively small part of the total surface area, as in Dermaptera,[26] Orthoptera,[26] Carabidae (Fig. 231, B).[29] These discrete epithelial pads are termed 'rectal glands' ; but all intermediate stages exist between them and a uniform epithelium.[15] Perhaps they are an adaptation permitting distension of the rectum.[42] Rectal glands become most obvious when the

rectum is a capacious sac; in the adults of Siphonaptera and Diptera [28, 89] they form large conical papillae (Fig. 231, D); in Lepidoptera they consist of numerous projections each made up of two or three large cuneiform cells.[27] Sometimes, in the course of development, other cells from the body cavity apply themselves to the outer surface of the epithelial pads, and produce a two-layered structure with a cavity between, as in the bee (Fig. 231, C) [69a, 232] and in *Chrysopa*.[146] They always have a rich tracheal supply, indicating a high rate of metabolism.[42]

The cuticle in the rectum is sometimes thinner than in the ileum, as in the Syrphid larva, *Syritta* [122]; and that over the rectal glands may be particularly thin: in *Carabus* it is only 1·5μ thick, being 3μ elsewhere in the rectum.[29]

Function—The mid-gut is separated from the hind-gut by a 'pyloric sphincter'; when this is closed the hind-gut receives only the contents of the Malpighian tubes (p. 305). Normally the sphincter opens from time to time and admits a portion of the mid-gut contents. When these enter they are quite fluid, and the most obvious function of the hind-gut in many insects is the absorption of water. In *Tenebrio* and other beetles,[80], Thysanura, Dermaptera, Orthoptera, Neuroptera, &c.,[255] the contents become progressively drier as they pass along the hind-gut. This desiccation is particularly evident in the rectum, where the material is in contact with the high epithelium or with the rectal glands; and here it is converted into a more or less dry pellet before it is discharged through the anus. The rectal glands probably play an important part in this process of absorbing water (Fig. 232).[255] In the larvae of Lepidoptera the hind-gut consists of a variable number of chambers separated by sphincters.[27] In *Vanessa* there are three such chambers, the ileum, the colon, and the rectum,[97] and the gradual drying of each bolus of moist residue into a faecal pellet as it passes through them is very obvious.[234]

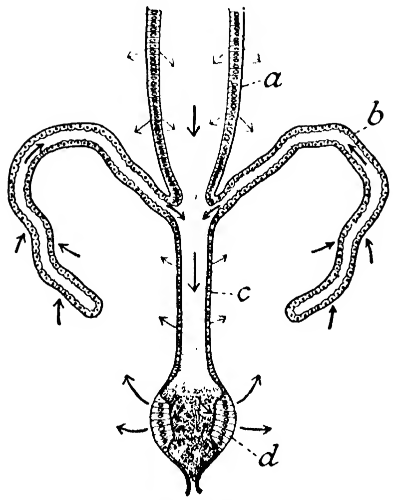


FIG. 232.—Diagram of water-circulation in alimentary and excretory systems of an insect (*after* WIGGLESWORTH)

a, mid-gut; *b*, Malpighian tubes; *c*, hind-gut; *d*, rectal glands.

In those insects, such as the adults of Diptera, Siphonaptera, Lepidoptera and Hymenoptera, in which the rectal contents are always fluid, the absorption of water is less apparent; but as we shall see in discussing excretion (p. 299) there is some evidence that the rectal glands are engaged in reabsorption here also, though it is possible that this is not their only function.[89]

In wood-feeding termites and Lamellicorn beetles [246] the hind-gut is very large, and one segment is enormously dilated; as we shall see later (p. 281) this appears to be the chief site of digestion and perhaps of absorption. Whether other substances besides water are absorbed in the hind-gut of most insects is not known. Experiments with fats, dyes and iron salts have generally proved negative [213]; though the hind-gut of some beetles is said to be permeable to glucose and to some dyes.[88] In such blood-sucking insects as mosquitos,

Glossina, *Cimex*, &c., absorption is to all appearances complete in the mid-gut, and nothing enters the hind-gut but a little haematin. We shall refer later to the possibility of the hind-gut being concerned in the uptake of salts (p. 284).

The capacious rectal ampulla of the *Dytiscus* larva has quite a different function. It serves to hold the large quantities of water which are swallowed by this insect at the time of moulting ; just as the crop or mid-gut serve to receive the air swallowed by terrestrial insects (p. 256). And throughout larval life the contents of this ampulla appear to vary so as to compensate for the varying state of nutrition of the insect,[189] serving in this respect the same function as the abdominal air sacs of Muscid flies (p. 193).

The peritrophic membrane in the hind-gut—In those insects which possess a peritrophic membrane, this continues into the hind-gut and often forms a sheath in which the faecal pellets are invested. The cuticle of the hind-gut frequently bears small backwardly directed spicules, which probably assist in drawing back the membrane and its contents when peristaltic waves pass along it. In Muscid flies a complex muscular organ, or ' rectal valve ', divides the hind-gut into two segments ; it consists of a fold of cuticle bearing sharp spines, and is preceded and followed by circular muscles which act as sphincters (Fig. 233). During each movement of this organ short lengths of peritrophic membrane are seized and dragged towards the rectum.[89] The rectal papillae of these flies, which are also covered with spicules. have likewise been thought to grip the peritrophic membrane and draw it to the anus [15] or, by their churning movements, to tear the membrane into fragments before it is discharged.[69] In mosquito larvae, the membrane may occasionally extrude from the anus as an unbroken tube almost as long as the larva itself.

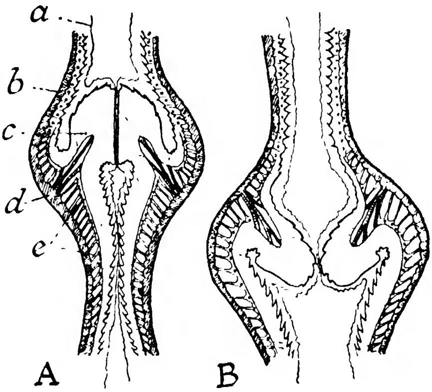


FIG. 233.—Rectal valve of *Calliphora* (*after* GRAHAM-SMITH). Two phases of its action are shown, to illustrate its function of drawing backwards the peritrophic membrane

a, peritrophic membrane ; *b*, muscular layer of hind-gut ; *c*, thick ' opalescent ' layer of the rectal valve with fibrillae ; *d*, point of attachment of muscles inserted into the valve ; *e*, epithelial cells.

The faeces—The excrement is often in the form of dry pellets, which are passed at more or less regular intervals by insects that feed continuously. If the cockroach is fed with soft food, such as a sweetened banana paste, this begins to enter the mid-gut within half an hour, and the passage through the entire alimentary canal requires from 9–33 hours.[206] The average time required for the passage in the silkworm is from 2–3 hours [203] and in the clothes moth *Tineola* 2–4 days.[228]

In other insects, such as Diptera, Hymenoptera and Lepidoptera, the excrement is always fluid, and it may be retained for long periods in the rectum : in the young honey-bee the rectum is not evacuated until foraging begins, that is, for about 3 weeks after emergence ; nor during the whole winter period. It is at such times that the reabsorption of water is probably important.[255]

In plant-sucking Hemiptera the excrement is a copious clear fluid. In

Aphids and Coccids it contains much unabsorbed organic matter, particularly carbohydrates, and furnishes the ' honey dew ' that collects on the leaves of plants in dry weather. Manna is a similar product from the Coccid *Trabutina mannipara* feeding on tamarisk in Sinai ; it contains 55 per cent. of cane sugar, 25 per cent. of invert sugar, and 19·3 per cent. of dextrin.[241] The significance of this wasteful method of feeding is generally supposed to lie in a deficiency of protein in the plant juice. The insect must therefore ingest great quantities to supply itself with protein, and has a large excess of carbohydrate to dispose of.[36] But there is little support for this view : the solid matter in the sieve tube juice of plants has a carbohydrate content of about 90 per cent. and protein about 5 per cent. ; while the honey dew, which varies in composition in the same Aphid feeding upon different plants,[53] contains some 85 per cent. of carbohydrate (sucrose 16·7 per cent., invert sugar 24·5 per cent., dextrin 39·4 per cent.) and 3 per cent. of protein.[229] Moreover, when *Trialeurodes* feeds on the same plants as Aphids, it has neither carbohydrate nor protein in its excrement.[242] It is possible that there is some necessary substance, other than protein, which can only be obtained in the requisite amounts by sucking excessive quantities of juice.

The excrement finds many uses in the economy of insects. The sugary excreta of Aphids and Coccids is much sought after as food by ants and other insects. The food residue is often utilized in constructing the cocoon : swallowed earth mixed with the Malpighian secretion hardens to form a cement for this purpose in *Cetonia* (Col.) larvae [246] ; the Javanese Tenebrionid *Platydema* spins its cocoon of threads made up of chitin fragments glued together, derived from the fungus on which it feeds.[198]

SECRETIONS OF THE ALIMENTARY CANAL

Salivary glands and their secretions—Of the dermal glands associated with the mouth appendages, the labial pair most commonly serve as salivary glands ; mixing their secretion with the food as this is taken in. They are of varied form,[114] and in some insects, notably in Hemiptera,[35, 74] they are made up of several lobes with quite different structure and presumably with different but unknown, functions.

The saliva is generally a clear watery approximately neutral fluid (*p*H 6· in the cockroach).[251] It is used in the first place for moistening the food when the cockroach feeds, its mouth parts are wet with the secretion ; whil Lepidoptera commonly extrude a drop of saliva in order to dissolve dried sugars &c.[214] In forms with piercing and sucking mouth parts, such as the Hemipter or the blood-sucking Diptera, in which the saliva is driven down the hypo pharyngeal or other duct, and poured out at the tip of the proboscis, it probabl serves to keep the mouth parts moist and clean in the intervals between feed ing.[130] But in many insects it contains active constituents.

Enzymes in salivary glands—Amylase and invertase are the enzym most frequent in the salivary glands. Amylase is exceedingly potent in th saliva of the cockroach ; it occurs also in *Calliphora*,[252] Aphids,[53] Jassids, &c. Invertase is present in Lepidoptera,[214] and in the cockroach *Blatel* (though not in *Periplaneta*).[251] In the larva of *Corethra*, the greater part digestion takes place in the dilated pharynx, the residue of the prey bei

ejected ; in this case the proteolytic digestive fluid appears to come from the salivary glands. Jassid bugs and some Aphids, when feeding on leaves, create localized white spots ; empty cells with their walls apparently intact being produced. The salivary secretion presumably diffuses through the cell walls without injuring them, decolorizing and destroying the chlorophyll. In some Coccidae (*Dactylopius*, *Aspidiotus*) the saliva (so far as can be judged from a histological examination of the leaves) appears to dissolve the cell walls.[205] Other examples of the digestive functions of the salivary glands will be considered under the heading of extra-intestinal digestion (p. 276). In Aphids [53] and Aleurodids [242] the track of the stylets as they pass between or through the cells, becomes walled off by a resistant sheath which is produced, at least in part, from the salivary secretion.

Saliva of blood-sucking insects—In the blood-sucking insects *Chrysops* (Tabanidae) [252] and the tsetse-fly *Glossina*,[252] the saliva contains no digestive enzymes ; but in *Glossina*, at least, a very active anti-coagulin is present.[263] If the glands are removed from the living tsetse-fly, feeding and digestion take place normally for some time ; but eventually the proboscis and the crop become blocked with clots of blood.[130] Anticoagulin is present in the salivary glands of some other blood-sucking insects, but not in all : it occurs in *Anopheles maculipennis* but not in *A. bifurcatus*, *Culex* or *Aëdes* [263] ; it occurs in *Philaematomyia* (though not in the non-blood-sucking species of *Musca*), yet it is absent in *Stomoxys* [46] ; it is present in the blood-sucking bug *Triatoma*, but apparently not in *Cimex* [46] ; and it is present in the salivary glands of *Gastrophilus* [59] although these larvae seldom seem to ingest blood.[187]

Salivary glands of the honey-bee—The honey-bee has a particularly complex system of salivary glands (Fig. 234). (i) A pair of *mandibular glands* with an acid secretion (pH 4·6–4·8) which are very active in the queen, less so in workers, and more or less vestigial in drones ; their function is not certainly known,[119] though perhaps their secretion serves to soften the cocoon at emergence.[61] (ii) The *pharyngeal glands*, which, in the early days of life of the adult bee, produce the ' royal jelly ' used for feeding the young brood and the larvae of queens. The bee requires pollen for these glands to become fully active, which happens at 3–6 days after emergence. Their secretion is again acid (pH 4·5–5·0).[119] Later in life, when the bee begins foraging at about 3 weeks after emergence, the pharyngeal glands begin to secrete the enzymes amylase and invertase, becoming most active in this respect in bees about one month

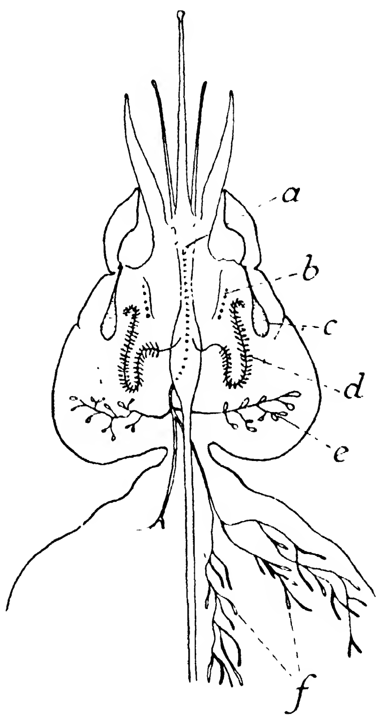


FIG. 234.—Salivary glands of *Apis*, schematic (*after* HESELHAUS)

a, opening of labial glands ; b, circumoral glands ; c, mandibular glands ; d, pharyngeal glands ; e, post cephalic part of labial glands ; f, thoracic part of labial glands.

old.[118] They are probably responsible for the invertase and amylase in the honey [119]; even honey made from pure cane-sugar syrup contains these enzymes.[193] (iii) The *labial glands*, each with two divisions : (*a*) the 'posterior cephalic glands' which produce a neutral secretion, apparently a fatty emulsion used for working the wax; (*b*) the 'thoracic glands' which are active throughout life, and produce a watery secretion, never containing any digestive enzymes and with a *p*H of 6·3–7, probably used in building the comb.[100]

Reaction of the intestinal contents—The digestive juices of most insects are weakly acid or weakly alkaline.[123, 221] Thus the *p*H of the mid-gut contents in the cockroach averages 6·2,[251] in *Dixippus* 7·0–7·5,[41] in various grasshoppers 5·8–6·9,[22] in Odonata 6·8–7·2,[202] in *Popillia* 7·4–7·5,[220] in *Chironomus* larvae 7·2–7·8,[50] in *Glossina* 6·5–6·6,[252] in *Apis* 5·6–6·3,[5, 109] and in *Apis* larva 6·8.[5] More acid conditions may sometimes result from bacterial fermentation, notably in the crop of Orthoptera [174, 213] in which the *p*H commonly ranges from 5·0–5·9.[22] This effect naturally varies with the diet: in the cockroach *Blatella* the *p*H in the crop falls to 4·4 after feeding with glucose which is readily broken down to lactic acid, 4·8 after lactose, 6·3 after protein.[251] Perhaps this is the cause also of the rather acid contents of the crop (*p*H 5·2) and mid-gut (*p*H 5·2) in termites.[180]

It is a characteristic of larvae of Trichoptera [202] and Lepidoptera [112, 219] that the mid-gut contents are always strongly alkaline (*p*H 9·0–9·4). Similar conditions are met with in some herbivorous Coleoptera [202]; but in the case of Lepidoptera it is interesting to note that this alkalinity is not confined to phytophagous species, for in *Galleria* feeding on honeycomb the *p*H of the mid-gut is 8·4, and in *Tineola* feeding on hair, &c., it is 9·9.[65]

In a few insects there are localized differences within the mid-gut. In *Tenebrio* larvae the anterior two thirds turn litmus red, the posterior third blue.[17] In *Lucilia* larvae the short middle segment of the mid-gut (p. 266) is strongly acid, with a *p*H of 3·0–3·5, while the segments which precede and follow this are about *p*H 7·5, except towards the hind end, where the contents become strongly alkaline (8·0–8·5) as the result of ammonia production (p. 301).[102] A similar region, with a *p*H of 3·0, occurs near the junction of the mid-gut and hind-gut in the wood-feeding termite *Zootermopsis*.[180] The nature of the acid in these cases is not known with certainty, but it is probably phosphoric acid [102]; in the normal bee the concentration of phosphate in the mid-gut contents (0·046 M) is nearly five times that in the blood (0·01 M), whereas the excreta contain only 0·005 M.[109] The reaction of the hind-gut will be considered in connexion with the urine (p. 301).

Digestive enzymes : adaptation to the diet—Broadly speaking the digestive enzymes in insects are adapted to the diet on which they feed. Omnivorous insects like the cockroach secrete protease, lipase, amylase, invertase and maltase, hydrolysing respectively natural proteins, fats, starch, cane sugar and maltose.[218, 251] The same series of enzymes appear in the mealworm [17] and in the phytophagous Orthoptera [11, 195] and Coleoptera [29] (though amylase is occasionally absent, as in *Popillia* [220]) in the larvae of Lepidoptera [203, 214] (though here again amylase is said sometimes to be wanting, as in *Laspeyresia* [219] and in some races of *Bombyx mori* [203]), and in the honey-bee.[70, 166] These enzymes are secreted, as we have seen, chiefly by the mid-gut and its caeca,

though invertase and amylase are occasionally contributed by the salivary glands (p. 272).

Insects which live on food rich in some particular substance generally produce the appropriate enzymes in particular abundance; and in those which live on a highly restricted diet, the enzymes present are correspondingly limited. Thus amylase is exceptionally active in the saliva of the cockroach.[195] In the bee, invertase first appears in the pharyngeal glands (p. 273) when the young bee starts foraging; it is already present in the mid-gut of the newly emerged bee, but later it increases greatly in amount.[118] Protease and lipase predominate in most carnivorous and necrophorous beetles [29]; in the predatory Carabids lipase is weak, carbohydrases are quite absent except a very feeble amylase, but proteases are extremely active.[195] Among Orthoptera, protease and lipase predominate in the predatory *Decticus*, in which lichenase acting on stored cellulose is absent; whereas carbohydrases, including lichenase, are very active in Acridiidae.[195] We shall discuss in a later section (p. 277) the distribution of enzymes which attack the skeletal substances of plants and animals.

These differences according to the composition of the diet are sometimes very evident when the developmental stages of an insect are compared. We have seen the wide range of enzymes present in caterpillars. In adult Lepidoptera, on the other hand, (*Deilephila*, *Macroglossa*, &c.,[214] *Laspeyresia*,[219] *Ephestia* [161]) only invertase remains (in saliva and mid-gut); and in insects such as *Dicranura*, *Lymantria*, &c., which have vestigial mouth parts, and which take no food, even invertase is wanting.[214] Larvae of the blow-fly *Lucilia* are adapted primarily to feed on meat; the mid-gut secretes protease and lipase; carbohydrases are absent save a very feeble amylase in the salivary glands.[102] Whereas in the adult blow-fly *Calliphora*, protease is relatively feeble, while amylase is active in the saliva and mid-gut, and invertase and maltase in the mid-gut.[252]

Calliphora, feeding largely on sweet substances, forms a marked contrast with *Glossina*, feeding exclusively on blood. In *Glossina*, protease is very active in the mid-gut, carbohydrases are absent except a very feeble amylase in the same region.[252] Whereas *Chrysops* (Tabanidae), which feeds on both blood and nectar, occupies an intermediate position: an active protease and invertase and a weak amylase are present in the mid-gut.[252]

The enzymes increase in quantity in the mid-gut in the early stages of digestion; they are clearly produced under the stimulus of feeding. Later they decrease; and they diminish towards the end of the intestine, being practically absent from the contents of the hind-gut; their fate is not known.[195] Attempts have been made to alter the relative activity of the enzymes in the cockroach by feeding it on diets with different proportions of protein and carbohydrate, but so far with negative results.[195]

Properties of the enzymes—In general, the digestive enzymes of insects resemble those of other animals: they are activated and inhibited by the same reagents, they are similarly affected by changes in *p*H and so forth.[251] The amylase in the saliva of the cockroach, for example, is inactivated if it is freed from chloride by dialysis, like the corresponding enzyme in human saliva [251]; the lipase has properties similar to that of vertebrates, &c.[195] But they are usually adapted to work best at the hydrogen ion concentration normal in the insect: the amylase of the cockroach, with its acid crop, has

an optimum *p*H of 5·9 [251]; that of the silkworm, with its very alkaline mid-gut, has an optimum *p*H of 9·5.[203]

The protease, like that of vertebrates, is made up of several components: a *proteinase* acting upon natural proteins, and a group of *peptidases* by which the products of protein digestion are further hydrolysed. The proteinase is always of the tryptic type; enzymes acting like pepsin in a strongly acid medium do not occur in insects. The proteinase of the cockroach,[251] tsetse-fly,[252] and blow-fly larva,[102] when acting upon gelatin, has a *p*H optimum of about 7·5; but it remains active well on the acid side of neutrality. In *Stenobothrus* and *Tettigonia* (Orth.) it has an optimum at 6·2 [195]; in the silk-worm, with its alkaline juice, at *p*H 9·5.[202] It is activated by 'enterokinase' extracted from the small intestine of the pig; but no activator of this type can be demonstrated in any part of the gut of insects. It is inhibited by 'zoo-kinase' or reduced glutathione, behaving in these respects like the trypsin of vertebrates.[195]

The proteinase liberates peptones, polypeptides, and some free amino-acids. The first two provide the substrate for the peptidases: (i) carboxy-polypeptidase, which attacks the peptide chain from the –COOH end, and depends for its action upon the presence in the chain of tyrosin or certain other specific amino acids, (ii) aminopolypeptidase, which attacks the chain from the NH_2– end, and will attack chains made up of any natural amino acids, and (iii) dipeptidase, which hydrolyses all dipeptides. These enzymes have been demonstrated in Carabids,[195] Orthoptera,[195] and caterpillars.[65] But whereas the proteinase is active in the contents of the gut, the peptidases occur much more abundantly within the epithelium; this suggests that the final hydrolysis of proteins may take place within the cells.

Digestive enzymes in the Malpighian tubes—It is generally accepted at the present time that the Malpighian tubes, which open at the junction of the mid-gut with the hind-gut, are excretory organs (p. 305). In many insects their secretion never mixes with the mid-gut contents; and by gross tests digestive enzymes are absent from them.[60] It was observed in the beetles *Gnaptor* and *Necrophorus*,[88] that although extracts from the Malpighian tubes could not themselves digest protein, they could facilitate protein breakdown by the mid-gut secretion. The explanation of this appears to lie in the fact that while the Malpighian tubes do not secrete proteinase, they contain varying quantities of peptidase, particularly dipeptidase (in Carabids and Orthoptera).[195] These enzymes, as we have seen, are mainly intracellular; it is therefore uncertain whether those in the Malpighian tubes are concerned in intestinal digestion, or merely in intermediary or cellular metabolism.

Extra-intestinal digestion—Since the saliva is usually ejected during feeding, a certain amount of digestion must commonly take place outside the body; notably in the plant-sucking Homoptera (p. 273). But in some insects solid foods of all kinds are to a large extent predigested in this way and absorbed in fluid form. The larvae of the Chalcid *Mormoniella* live as ectoparasites between the wall of the puparium and the pupa of Muscid flies, perforating the pupal integument, digesting and absorbing the tissues.[47] The larva of the wasp *Pseudagenia*, which feeds on spiders, emits a digestive fluid which causes complete solution not only of the protein contents but of the chitin.[179] *Cryptochaetum* (Agromyzidae),[225] and other internal

parasites, contain no solid matter in the gut in their young larval stages; some extra-intestinal digestion of the tissues probably occurs. When the larvae of ants, or of the hornet *Belonogaster*, &c., are fed, they discharge a strongly proteolytic secretion. This fluid, which has a somewhat sweet taste, is greedily eaten by the nurse; and it has been suggested that such exchange of nourishment may form the basis of the social habit.[248] Other examples are the larva of *Syrphus pyrastri*, which preys on Aphids,[128] the larva of *Miastor metroloas*, which pours out a secretion, probably from the salivary glands, which causes solution of some of the constituents of wood,[208] and the predaceous Heteroptera. In the case of the predaceous Pentatomid *Troilus* even the cuticle is said to be dissolved.[241]

In many of these cases there is some doubt whether the digestive fluid comes from the salivary glands or from the gut. But some of the most striking examples of extra-intestinal digestion occur in beetles, in which salivary glands are wanting. In the larva of *Dytiscus* the contents of the gut are regurgitated to the oesophagus and through the perforated mandibles. As this larva feeds on some transparent insect, a black fluid can be seen to come from the tips of the jaws and spread among the organs. At once the larva is paralysed and dies; the secretion appears to act as a nerve poison, making the prey quiescent. Very quickly the tissues melt away into a liquid with floating granules, which can be seen flowing back into the mandibular hooks.[176] A caddis larva 12 mm. long may be completely emptied in 10 minutes.[20] A similar process occurs in *Hydrophilus*, *Myrmeleon*, *Chrysopa*, &c.[128]; and a large part of the digestion in Carabids,[113, 120] Cicindelids,[254] and the larvae of *Lampyris* feeding upon slugs,[238] takes place outside the body by ejection of the stomach juices from the mouth. In the larvae of *Lucilia* and *Calliphora*, the proteolytic enzymes persist in the excreta and are responsible for some liquefaction of the meat, even in the absence of bacteria.[102, 261]

The softening of the cocoon by means of fluids ejected at the time of emergence forms a special type of extra-intestinal digestion. The newly emerged silkworm moth extrudes a liquid containing a very active protease capable of attacking the sericin layer of the silk fibres[108]; the origin of this fluid is uncertain. Imagines of *Dicranura* and other Lepidoptera emit from the mouth a strongly alkaline fluid, containing some 1·4 per cent. of potassium hydroxide, which appears to come from the gut.[127]

DIGESTION OF SOME SKELETAL AND OTHER SUBSTANCES OF PLANTS AND ANIMALS

In the section on digestive enzymes, we have considered the mechanisms of digestion for the ordinary constituents of the food. Here we shall discuss the adaptations to deal with special substances.

Digestion of leaves and pollen—Great quantities of the foliage of plants are devoured by insects, but for the most part the cell walls and skeletal parts pass through the gut apparently unchanged. The fragments of leaves ingested by caterpillars often appear in the faeces with the cell contents still intact, except at the margins where the cells have been actually cut open.[3, 174] But sometimes the contents of the cells can be completely digested without mechanical or chemical breakdown of the walls; in *Gastropacha rubi* quite

thick pieces can be emptied in this way [19]; and we have referred already to the phenomenon in plant-sucking forms (p. 273). The same change is seen in pollen in the stomach of the bee: some pollen grains pass through unchanged; the contents of many are completely dissolved; yet none are crushed. Here digestion may take place perhaps through the micropylar membranes.[249] Starch grains often escape digestion unless broken open; but the pollen starch is easily digested by the bee; perhaps it lacks a protective sheath.[137]

Enzymes capable of attacking cellulose have not been found in phytophagous insects,[195] but hemicellulase seems to be present in the gut contents of *Forficula*, and various Acridiids,[19] and in the fluid from the crop of *Dixippus* [14]; and lichenase, producing glucose from lichenin or stored cellulose, occurs in *Stenobothrus*, *Tettigonia*, &c.[195]

The chlorophyll of plants is partially broken down in the gut of the silkworm and other caterpillars. Phytol and methoxyl are removed and perhaps utilized by the organism, and a crystalline derivative named 'phyllobombycin' can be isolated from the faeces. The breakdown does not extend to the liberation of porphyrins as it does in ruminants.[77] Complicated changes are described in the chlorophyll in the gut of *Vanessa* larvae, leading it is claimed to the production of a red pigment [133]; but these changes have not been studied chemically.

Digestion of wood—The chief constituents of wood are cellulose (comprising 40 to 62 per cent. of the dry weight) and lignin (18 to 38 per cent.). Hemicelluloses, a mixture of polysaccharides, both hexosans and pentosans, come next. Pentosans (6 to 23 per cent.) yielding xylose and arabinose on hydrolysis, occur chiefly in the cell walls; hexosans (2 to 14 per cent.) yielding glucose, chiefly as reserve material. Starch ranges from 0 to 5·9 per cent.; sugar, expressed as glucose, from 0 to 6·2 per cent.; protein from 1·1 to 2·3 per cent.[144]

Of these constituents, the lignin seems never to be digested by insects. Termites feeding on wood containing cellulose 54·6 per cent., pentosans 18·0 per cent., lignin 27·4 per cent., produced faecal material containing cellulose 18·0 per cent., pentosans 8·5 per cent., lignin 75·5 per cent.[180] Here an extensive digestion of cellulose has taken place. As we shall see later (p. 282) termites are dependent for this activity upon the protozoal fauna in the gut; they produce no cellulase in their own secretions. The same is true of the wood-feeding cockroach *Cryptocercus* (p. 282) [230]; and the larvae of Lamellicorn beetles apparently depend on the cellulose-digesting bacteria in the food [246, 250]; though it is uncertain to what extent cellulose is in fact broken down by them (p. 281).[185] On the other hand a true cellulase, producing glucose from filter paper, secreted by the mid-gut of the insect itself, occurs in the larvae of some beetles: *Cerambyx cerdo*,[185] *Hylotrupes bajulus*,[73] and *Macrotoma palmata* [144] (Cerambycidae), and in *Xestobium rufovillosum* (Anobiidae).[185] The cellulase in the gastric juice of *Stromatium* larvae (Col.), with a *p*H optimum of 5·5, will attack cellulose even when bound with lignin as lignocellulose.[144] Cellulose and pentosans are present in the frass of *Xestobium* in much smaller amounts than in the original wood [40]; the cellulose/lignin ratio varied from 2·38 to 2·81 in sound oak wood on which the larvae were feeding; it was between 0·86 and 1·24 in the frass; so that assuming

no lignin was attacked, one-third of the total weight ingested has been assimilated, and 80 per cent. of this was cellulose.[160] The wood adjacent to the borings (with a ratio of 1·13) may be almost as much affected as the frass, suggesting that the enzyme is still active in the excreta.

But many wood-boring insects cannot digest cellulose. In the case of *Lyctus*,[40] *Xystrocera* (Col.),[144] and *Cossus* (Lep.) [185] there is no difference between the cellulose content of the food and excrement. Hemicellulases are probably more widespread: the Cerambycid larva *Phymatodes variabilis* contains a 'xylanase' hydrolysing xylosan to xylose, and thus reducing the pentosan content from 23·54 per cent. in the beech wood on which it feeds, to 18·48 per cent. in the excreta.[200] The larva of *Cossus* secretes a 'lichenase' acting on reserve cellulose [144]; but in *Lyctus* neither cellulase nor hemicellulase occurs.[165]

Where cellulose is not attacked the insects become increasingly dependent on the starch and sugar in the wood. *Cossus* larvae must make great burrows and ingest huge quantities of wood in order to obtain sufficient carbohydrates: there were 2·27 per cent. of reducing sugars in poplar wood on which these larvae fed, none in the excreta.[185] Assimilable carbohydrate is probably a limiting factor in the rate of growth, for when fed on beetroot they will complete their development in one year instead of three.[185] Egg-laying females of *Lyctus* 'taste' and select wood in which starch is present in the cells; wood slowly seasoned, and so free from starch, is immune against attack.[260] If the wood is extracted with water at 60° C., so that sugars are removed but starch remains, *Lyctus* larvae can live but fail to grow; if the starch also is extracted, by using boiling water, the larvae die; but growth takes place if sugar is restored.[165] The Cerambycid larva *Macrotoma palmata* which, as we have seen, contains an active cellulase, can invade the heartwood of trees with a starch and sugar content of only 0·47 to 0·7 per cent.; whereas when the Cerambycid *Xystrocera globosa*, from which cellulase is absent, attacks these same trees, it is found only in the sapwood with 10 per cent. of starch and sugar.

Digestion of collagen, silk and keratin—These animal proteins are very resistant to most digestive enzymes. Collagen, the main constituent of fibrous tissues, is completely unaffected by proteinases of the tryptic type. In the blow-fly larva *Lucilia*, however, there is a distinct enzyme, a 'collagenase', separable from the tryptase, which can digest collagen and elastin in alkaline solution (optimum *p*H 8·5). This enzyme is present in the excreta of bacteria-free larvae, and is doubtless of importance in the extra-intestinal digestion of the fibrous septa of the muscles.[102] Silk is digested by the various clothes-moth larvae, and by the larvae of *Anthrenus*, but nothing is known of the enzymes concerned.[2]

Keratin, the chief constituent of hair and feathers, forms the basis of the diet in the clothes moths, in *Anthrenus* and other Dermestids, and in Mallophaga. Its digestion in *Tineola* is evidenced by the fact that the excreta contain 4·0–4·6 per cent. of sulphur, whereas the wool on which they feed contains 2·2–2·6 per cent.[228]; yet extracts from the gut of clothes-moth larvae are without effect on keratin.[196] That is because the keratinase system is inhibited by oxygen. Keratin is believed to be made up of long peptide chains, folded so as to allow elasticity; and these are bound together by

S—S linkages, the two halves of a cystine molecule being shared by two adjacent chains. The peptide linkages cannot be attacked by hydrolytic enzymes until these S—S bonds have been broken by reduction and the chains set free. In the mid-gut secretion of *Tineola*, *Anthrenus*, and Mallophaga, there is a strong reducing agent of unknown nature capable of opening these S—S bonds; the protein is then digested by the proteinase. Most proteinases of the tryptic type are markedly inhibited by –SH compounds; but the proteinase of these insects is peculiar in being insensitive in this respect. It is these two adaptations, the low oxidation-reduction potential of the medium, and the low sensitivity to –SH compounds, which enable these insects to digest keratin.[134] It is interesting to note that *Galleria* larvae secrete a proteinase which is less affected by –SH compounds than is the trypsin of vertebrates; if a suitable reducing agent is added to the extract, it also is capable of digesting keratin.[65]

Digestion of wax—The diet of *Galleria* larvae, which feed on honey comb, consists largely of wax. The honey comb contains about 60 per cent. of wax, the excreta only about 28 per cent.[148]; part of the wax is certainly utilized. This fraction is variously estimated as 34–43 per cent.[64] and 50 per cent.[58] It is believed to comprise all the alcohol components of the wax, a part of the fatty acids and esters of high molecular weight, but none of the paraffins.[64] The lipase extracted from the gut of the larvae will act on tributyrin, olive oil, &c., but has no action on 'myricin', i.e. the esters of higher alcohols (p. 330).[64, 121] Possibly the breakdown of some of the components is begun by the bacteria present in the gut.[58]

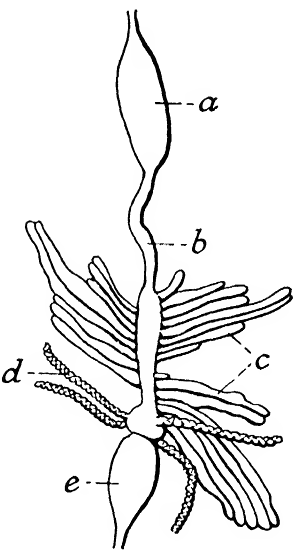


FIG. 235.—Gut of *Aphanus* (Lygaeidae) showing bacterial crypts (*after* KUSKOP)

a, dilated segment of mid-gut; *b*, narrow segment of mid-gut; *c*, bacterial crypts; *d*, Malpighian tubes; *e*, rectum.

THE ROLE OF LOWER ORGANISMS IN DIGESTION

Bacteria—The gut of many insects contains a bactericidal principle of unknown nature, which greatly restricts the bacterial flora.[62] Thus in the gut of the blow-fly larva *Lucilia*, the common saprophytic organisms are wanting (perhaps they are killed off in part in the acid segment of mid-gut (p. 274)) and the flora is limited to a few kinds of aerobic bacilli. These are non-proteolytic, and are therefore of no importance in digestion. Digestion takes place equally well in larvae reared from sterilized eggs in the absence of bacteria[103]; though in the early stages of feeding on muscle, the alkalinity produced by bacteria is of some importance in liquefying the tissue.[103]

Yeasts capable of breaking down starch and sucrose, are present in the gut of Jassids, and are ejected with the saliva during feeding.[98] In those Hemiptera-Heteroptera which feed on plant-juices (Pentatomids, Coreids, Lygaeids, &c.) there are numerous caeca in the mid-gut (Fig. 235) which are uniformly inhabited by bacteria of varied form, each characteristic of its own host

species [87, 124]; but there is no evidence that these play any part in digestion. The same applies to the bacteria in the gut of the blood-sucking Reduviidae (Fig. 236); for these form their colonies in the dilated segment of the mid-gut where digestion does not occur (p. 266)[257]; and it applies to the bacteria in the mid-gut caeca of Trypetidae.[210]

Bacteria may be of importance in the breakdown of some of the constituents of wax by larvae of *Galleria*,[58] yet these larvae can be reared under sterile conditions.[261] Bacilli and cocco-bacilli capable of oxidizing paraffins occur in the mineral oils of California. It is doubtless the presence of these organisms which makes possible the colonization of the petroleum wells by larvae of the Ephydrid, *Psilopa petrolei*; the organisms in question are abundant in the gut of this larva.[226]

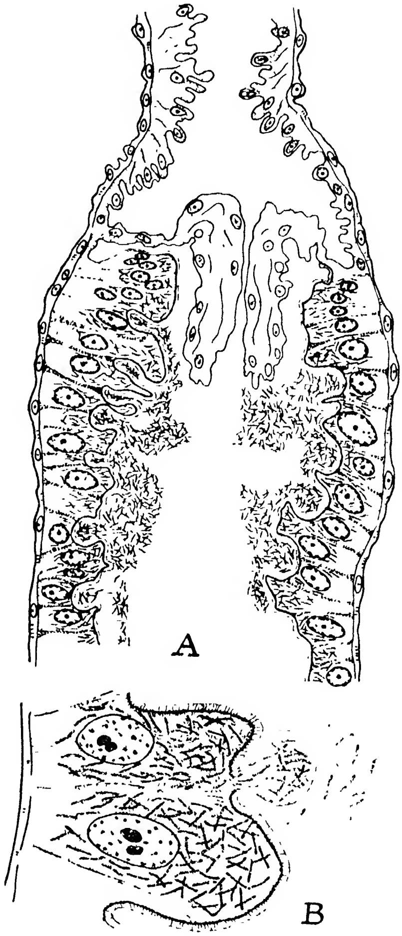


FIG. 236

A, longitudinal section of the junction between fore-gut and mid-gut of 2nd-stage nymph of *Rhodnius*, showing bacteria in the mid-gut cells and free in the lumen; *B*, detail of mid-gut cells showing discharge of bacteria. (*After* WIGGLESWORTH.)

Bacterial fermentation chambers—In the digestion of cellulose by many mammals, bacteria play an important part; but in most phytophagous insects, as we have seen, the food is passed through the gut so rapidly (p. 271) that no appreciable fermentation can take place. Cellulose-fermenting bacteria are, however, always present in rotting wood and vegetation, and must form an important element in the diet of insects feeding on such materials. In some insects they may continue their action in the gut. In the hind-gut of Lamellicorn larvae there is a dilated sac, the ' fermentation chamber ' which is probably important for this purpose (Fig. 237). On the walls of this chamber are areas of cuticle bearing branched spines; between these areas the cuticle is thin and pierced by fine canals; this chamber is regarded as being the chief site of digestion and absorption.[149] The woody particles may be held up for weeks within this sac, to be fermented by the bacteria. The enzymes secreted in the mid-gut accumulate here also, and ultimately digest the dead bodies of the bacteria, and of the flagellates which feed upon them.[250] Larvae of *Cetonia*, feeding on the decaying pine needles of ant heaps, are said to grow only at those temperatures at which the bacterial mixture present can ferment cellulose.[246] On the other hand, in the larvae of *Dorcus* and *Osmoderma*, although the fermentation chamber is full of bacteria, no evidence of cellulose breakdown could be obtained.[185] Certain Tipulids, also, have fermentation chambers in the hind-gut [34];

whereas the wood-feeding cockroach, *Panesthia*, depends for the decomposition of its cellulose on bacteria in the crop.[44]

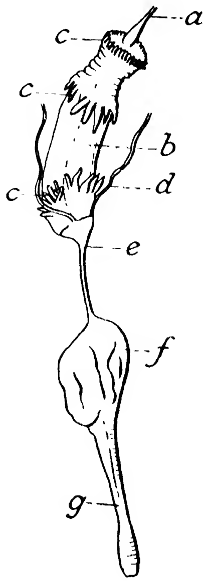


FIG. 237.—Gut of larva of *Oryctes nasicornis* showing the bacterial fermentation chamber in the hind-gut (*after* MINGAZZINI)

a, oesophagus ; *b*, mid-gut ; *c*, rings of mid-gut caeca ; *d*, Malpighian tubes ; *e*, hind-gut ; *f*, fermentation chamber ; *g*, rectum.

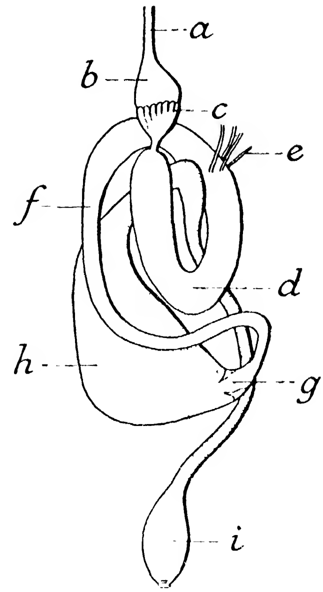


FIG. 238.—Gut of *Eutermes* showing rectal pouch (*from* WEBER *after* HOLMGREN)

a, oesophagus ; *b*, crop ; *c*, proventriculus ; *d*, mid-gut ; *e*, Malpighian tubes ; *f*, hind-gut ; *g*, rectal valve ; *h*, rectal pouch ; *i*, terminal segment of rectum.

Protozoa—In wood-feeding termites, although bacteria are present,[13] intestinal protozoa are the chief agents in the breakdown of cellulose (though it has been claimed that these protozoa themselves contain the bacteria which are ultimately responsible [173]). The hind-gut is enlarged to form a pouch which is always teeming with many kinds of flagellate protozoa (Fig. 238). These take up and digest particles of wood, different species perhaps playing a different part in the process. Such termites can live for long periods on a diet of pure cellulose ; but if they are deprived of their protozoa by starvation, by exposure to a high tension of oxygen (3·5 atmospheres of oxygen) or by a high temperature (24 hours at 36° C.) this ability is lost, and they die in 3–4 weeks if given a diet of wood. But they can still live on fungus-digested cellulose ; and if their protozoal fauna is restored they can live on wood again.[43] It has been calculated that, of the material normally absorbed from the intestine by *Zootermopsis*, about two-thirds has been rendered assimilable by the digestive activity of the protozoa.[111] The wood-feeding cockroach *Cryptocercus*, also contains abundant cellulose-digesting flagellates ; and cellulase can be extracted from the hind-gut where the flagellates are confined ; as it can be from the flagellates of termites. But cellulase is absent from insects that have been freed of protozoa by high temperature or oxygen pressure.[230]

Fungi—Insects sometimes make use of fungi in the conversion of woody materials to an assimilable form. These organisms may grow outside the body,

and themselves provide the immediate source of nourishment. Certain ants and termites maintain 'fungus gardens' cultured upon fragments of leaves or the comminuted vegetable matter in the excrement. The egg-laying females of bark beetles (Scolytidae) regularly infect their burrows with moulds ('ambrosia fungi') which grow upon the dead wood [83]; but though these moulds are frequently eaten they do not appear necessary for the normal development of the larvae.[92] The saw-fly *Sirex* also transmits a fungus which infects the burrow, but instead of forming a dense outgrowth on the wall like the ambrosia fungus, it penetrates the surface layers of the wood.[33] Larvae of *Sciara* which burrow into wood infected with dry rot, feed not on the wood but on the fungus.[12]

NUTRITION

Water requirements—The amount of water needed in the food depends upon the rate at which it is lost by the body; and this depends, on the one hand, upon the properties of the cuticle (p. 20) respiratory system (p. 190) and excretory system (p. 299), and on the other, upon the drying power of the air (p. 355). Insects such as the honey-bee or Muscid flies, which produce liquid excrement, must drink frequently if they are to survive; whereas insects such as the mealworm, which extract almost every trace of water from their excrement, can live on very dry materials. Such insects can cover much of their water requirements by the water produced in metabolism from the oxidation of the dry food (p. 357). But since foodstuffs are mostly hygroscopic, completely dry foods can exist only in a completely dry atmosphere; and it becomes difficult to separate these two factors.[38] Insects in deserts are able to obtain water by eating fragments of dead vegetation which have absorbed water from the atmosphere during the night, when the temperature falls and the relative humidity is high.[37] And there is some evidence that mealworms may eat excessively, and pass large amounts of undigested food, in order to benefit from the small proportion of water it contains.[197]

Salt requirements—It is possible that salts may constitute a limiting factor in the growth of insects upon some diets. In the larva of *Tribolium* (Col.), phosphorus composes about 0·19 per cent. of the wet weight at all stages; growth is delayed if the phosphorus content of the flour is below 0·1 per cent.[156] Phosphorus may also be a limiting factor in the growth of *Lucilia* larvae on mammalian blood.[104] *Drosophila* has been reared on a diet containing K_2HPO_4 and $MgSO_2$ as the sole salts; NaCl and $CaCl_2$ being present only in traces as impurities. The potassium and phosphate were essential, and no flies were raised if sodium was substituted for potassium; a fly occasionally developed with potassium phosphate alone.[136] But it is not known what the larvae contrived to pick up from the impurities, for no analysis of the larval ash was made. Adult Lepidoptera frequently drink water where this is contaminated with excrement or sweat, and will suck up sweat from the skin.[162] It is possible that this habit may be connected with salt requirements.

Uptake of salts by aquatic insects—The thin-walled anal papillae of Culicid and other Nematocerous larvae are, as we have seen, of little importance in respiration (p. 197). They are permeable to water and to salts; and water is in fact continuously taken up by them into the blood and continuously

eliminated by the Malpighian tubes (p. 299).[256] But their most important function appears to be the uptake of chloride ions.[116] If *Culex* larvae have the chloride content of their blood, expressed as NaCl, reduced to 0·05 per cent. by keeping them in distilled water, and they are then transferred to tap water containing less than 0·006 per cent. of NaCl, they can absorb the chloride, and raise the concentration in the blood to the normal 0·3 per cent. If the larvae are reared in water with a very low chloride content, the anal papillae become greatly hypertrophied (Fig. 239). The mosquito larva *Aëdes aegypti*, which lives in small collections of clean rain water, is much more efficient at collecting chloride in this way than *Culex pipiens*, which commonly develops in contaminated water. The chloride concentration in the water can be a limiting factor in growth: *Aëdes aegypti* can grow in water with a smaller chloride content than *Culex pipiens* can.[258] The relative efficiency of this mechanism may perhaps be the factor which limits the breeding of certain species of *Anopheles* to slightly brackish waters.

It is probable that many other thin-walled outgrowths of the body wall in insects will prove to have this same function; such organs as the anal gills in larvae of *Eristalis* and other Syrphids [211] or in *Pantophthalmus*,[227] the numerous ventral papillae of Blepharocerid larvae, the gills of Trichoptera, &c. We discuss elsewhere the possibility that the rectal glands may be concerned in reabsorbing chloride from the excreta; there is the further possibility that in such aquatic insects as the larvae of *Aeschna*,[75] Ephemeroptera, *Corixa*,[175] &c., in which water is constantly passed in and out of the rectal chamber, the enlarged cells corresponding to the rectal glands may be absorbing chloride or other ions from the environment.

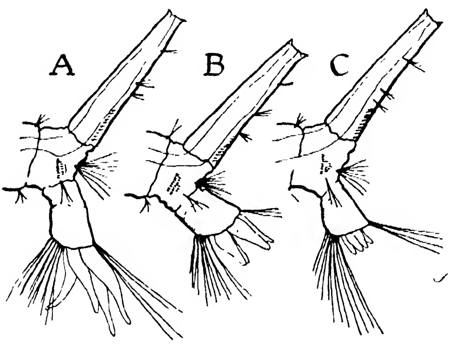


FIG. 239.—Posterior extremity of larvae of *Culex pipiens* showing variation in size of anal papillae (*after* WIGGLESWORTH)

A, reared in distilled water; *B*, in tap water with a chloride content equivalent to 0·006% NaCl; *C*, in salt water equivalent to 0·65% NaCl.

Nutrition through the body surface—In Nematocerous larvae it is not known whether substances other than chlorides are absorbed through the cuticle [129]; but some parasitic insects obtain all their nourishment in this way. In larvae of Strepsiptera,[154] the early stages of many Tachinid larvae (e.g. *Thrixion* [164]), the first stage larvae of some Hymenopterous parasites and in *Cryptochaetum* (Agromyzidae) [225] there is no buccal opening. It may be that the 'tails' of many parasitic larvae, or the terminal ampulla of Braconids (p. 204) are specially concerned in the uptake of salts or other food substances. In *Cryptochaetum* the permeability of the tails for water and salt resembles that of the anal papilla of mosquito larvae.[225]

Organic substances are required for growth and reproduction on the one hand and for energy production on the other. It is characteristic of many insects that, if given a diet adequate for the second purpose, they may survive for a long time in an apparently normal state, but without making any growth. Cockroaches can maintain their body weight for many months on a nitrogen-

free diet[264]; termites can exist for considerable periods on pure cellulose; but for growth they must have a source of organic nitrogen, sulphur, phosphorus, and salts.[45]

Two factors determine the requirements for either purpose:

(i) The organic substances must either be directly assimilable by the tissues (like glucose or the natural amino-acids), or they must be susceptible of being hydrolysed to assimilable components by the digestive system. Cellulose or keratin are of value in nutrition only to those few insects which can break them down; albumen is of no benefit to adult Lepidoptera because they lack proteolytic enzymes.

(ii) The insect must be able to synthesize from the raw materials provided, all the complex organic substances in its body. Where it is incapable of carrying out such syntheses from the simple components of proteins, fats and carbohydrates, the complex elements themselves, or special substances which can serve as precursors for them, must be included in the diet. These are called accessory substances.

Available carbohydrates—The extent to which organic materials can be digested by insects has been dealt with in preceding sections. But one aspect

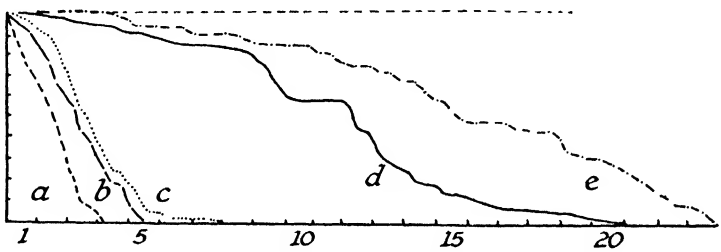


Fig. 240.—Survival of bees fed with different sugars (*after* Vogel)

Ordinate: percentage of bees surviving. Abscissa: days from commencement of experiment. *a*, fasting; *b–d*, received one-eighth part of 2M sucrose; in addition, *b* received seven parts 1M lactose; *c*, seven parts water; *d*, seven parts 1M sucrose; *e*, abundant M/2 sucrose.

of this question which may be considered here is the availability of various sugars as sources of energy. This has been tested by comparing the length of time insects can survive when given water alone or a mixture of sugar and water. The larva of the honey-bee can survive from 4 to 7 times as long when given sucrose, fructose, maltose, melizitose, dextrose or honey, than when given pure water. It can survive about 3 times as long on trehalose and dextrin, less than twice as long on lactose and galactose and no longer on glycogen or starch.[16] In the adult honey-bee, lactose is again found to be of no value in prolonging life[172]; the same is true of a number of other sugars and sugar alcohols which are tasteless to the bee (Fig. 240). Of the sugars and sugar alcohols commonly met with in nature, as nectar (sucrose, glucose and fructose) or honey dew (sucrose, glucose, fructose, melizitose, trehalose, mannitol, dulcitol, raffinose) all are utilized with the exception of dulcitol. It is worth noting that the tasteless pentose sugars xylose and arabinose are practically equal in value to cane sugar.[237] The adult fly *Calliphora* dies in 2–3 days at 26° C. if given water alone; it will live 1–2 months on water and cane sugar. Almost all other sugars are as effective as cane sugar with the exception of lactose and the pentose xylose, which enable

the fly to live 1–2 weeks, and cellobiose, which is of no value. These results are explicable on the assumption that enzymes hydrolysing α-glucosides and α-galactosides are present, together with a weak β-galactosidase (acting on lactose) but no β-glucosidase to act on cellobiose.[78]

Accessory substances—The amino-acids resulting from protein digestion are among the most important raw materials for growth. In mammals, certain of the amino-acids are essential in the sense that they cannot be synthesized from others and must be included in the diet. But it is not known which, if any, of the amino-acids are essential in insect nutrition. For although *Drosophila* larvae have been reared with ammonium salts,[136, 233] and the cockroach with the simple amino-acid glycine,[264] as the sole source of nitrogen, there is no reason to doubt that the numerous bacteria and yeasts present in the medium and the gut were responsible in these cases for synthesizing more complex substances.

Most accessory substances, provisionally termed vitamins, are not characterized chemically, but only by their sources and a few of their properties. But it is evident from what is known of these substances that the synthetic powers or the requirements of insects differ markedly, both among insects themselves, and when they are compared with other animals. Thus, ascorbic acid (vitamin C) is not required by *Drosophila* [11] or *Tribolium* [217]; and cockroaches (*Blatella*), reared aseptically for 15 years on a diet free from vitamin C, were found to contain the same amount of this substance as newly captured insects.[262] The fat soluble vitamin A is not required for growth by *Drosophila* [11, 126] or by *Lucilia*.[104] Nor is calciferol (vitamin D) required by these insects or by *Tribolium*.[156] On the other hand, *Lucilia* does need a fat soluble substance present in wool-wax and muscle oil which is certainly a sterol is perhaps cholesterol itself [104]; and a substance of similar distribution is required by *Ephestia* [183] and *Blatella*.[147]

Among the B group of water soluble vitamins the differences between different insects are apparent. Several factors present in yeast are required by *Drosophila*,[11, 93, 136] *Lucilia*,[104] *Tribolium*,[215, 217] and *Blatella*.[147] Vitamin B_1, among others, is required by *Lucilia*; but neither this nor B_2 is required by *Drosophila*.[126] Mammalian blood is an inadequate diet for *Lucilia*; it becomes adequate if phosphate is added,[104] together with vitamin B_1 and at least two heat-stable factors present in an autoclaved extract of yeast.[104] If *Tribolium* larvae are sufficiently provided with a heat-stable factor from autoclaved yeast, then progressive addition of rice polishings causes a progressive shortening of the time required for pupation.[215] Larvae of the mosquito *Aëdes* also require vitamin B_1, a heat-stable substance in yeast extract, as well as another factor, distinct from vitamin B_1, which is plentiful in rice polishings and other sources.[231]

Micro-organisms as sources of accessory substances—The eggs of insects can be sterilized, and the resulting larvae reared on sterile media. *Drosophila*,[85] *Calliphora*,[261] *Lucilia*,[104] and *Blatella* [262] have been so raised. But this is possible only if they are provided with all the necessary accessory factors. If these are deficient, infection of the food with micro-organisms (in the case of *Drosophila*, particularly the introduction of yeasts) improves the rate of growth.[12, 85] Sterile *Lucilia* larvae will grow on beef muscle; they fail to grow on guinea-pig muscle; but if this is infected with *Bacillus coli*

or if a yeast extract is added to it, normal growth takes place (Fig. 241).[104] *Lucilia* larvae cannot grow on sterile blood, but growth is much improved by the presence of bacteria.[104] A sterile blood medium is insufficient for larvae of *Culex* and *Aëdes*; it becomes adequate if infected with bacteria.[188] In these cases there is little doubt that the micro-organisms are synthesizing the necessary vitamins of the 'B' group.

We have considered the bacteria and protozoa in the hind-gut of Lamellicorn larvae and termites from the standpoint of digestion (p. 280). These organisms digest cellulose and utilize it for their own growth. The insect probably subsists to a great extent upon their dead bodies. They may therefore be regarded as synthesizing essential matter for their hosts, using as raw materials not only the cellulose-containing food, but excretory substances poured into the gut by the Malpighian tubes. It has even been suggested that the free bacteria in the gut of termites may utilize atmospheric nitrogen,[173]

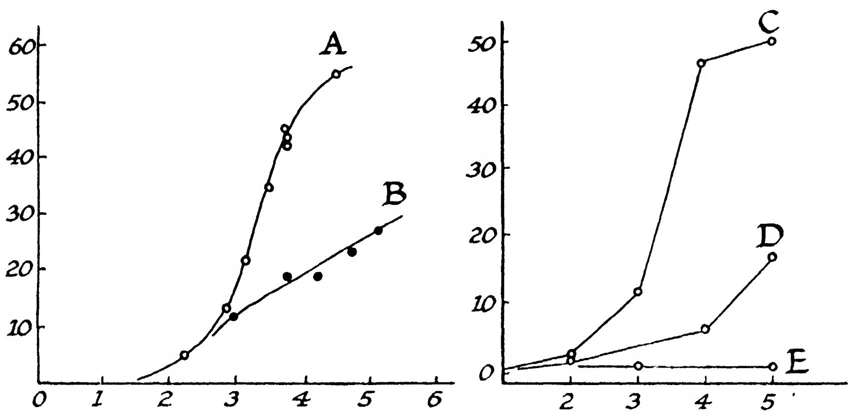


FIG. 241.—Accessory factors and growth in aseptic larvae of *Lucilia* (*after* HOBSON)

Ordinates: mean weight per larva in mg. Abscissae: days after hatching. *A*, grown on sterile guinea-pig muscle + aqueous yeast extract; *B*, on sterile guinea-pig muscle + equivalent amounts of yeast extract ash. *C*, grown on blood + marmite (autolysed yeast); *D*, blood + yeast extract; *E*, blood alone.

and the same suggestion has been made in regard to the caecal bacteria of the olive fly *Dacus*[167]; but at present there is no experimental basis for this idea.

Hereditary micro-organisms—Many insects contain specific micro-organisms, which are present in every individual, and are transmitted from one generation to the next by elaborate mechanisms (p. 406). Such organisms may be confined to the lumen of the gut, as in the mid-gut diverticula of Pentatomids, Lygaeids, &c.,[87, 124] and Trypetids.[210] They may occur in cells of the gut wall and be constantly set free into the lumen, as in the larvae of some Cerambycids,[95] in pouches of the mid-gut in Anobiids,[30, 95] in the blood-sucking Reduviids *Rhodnius*, *Triatoma*, &c.,[57, 257] and in Pupipara.[7, 265] In adult *Glossina* they are confined to special cells or 'mycetocytes' grouped to form a 'mycetome' in the wall of the mid-gut[186, 252]; and mycetomes in the gut wall occur also in *Anoplura*[9, 184] and *Camponotus* (Formicidae).[34] Modified Malpighian tubes form mycetomes in some species of *Apion* (Curculionidae)[159] and *Donacia*, &c. (Chrysomelidae).[212] But frequently the cells containing micro-organisms are entirely separate from the gut: mycetocytes are scattered singly throughout the fat body of cockroaches,[84] strings or masses of modified

fat cells form mycetomes in Aphids, Coccids, Aleurodids, Cicadids, &c. Mycetomes away from the gut, often connected with the fat body or the gonads, occur in *Cimex*,[32] *Formica fusca*,[132] and the beetles *Oryzaephilus surinamensis*,[115] *Sitodrepa panicea*,[115] *Lyctus*,[165] *Calandra* and *Rhizopertha* [143] among others.

These micro-organisms are of many kinds. In Anobiid and Cerambycid beetles they are evidently yeasts; and perhaps also in Aphids. In cockroaches,[86] Reduviids [257] and many other insects they resemble diphtheroid bacilli. In some they appear unlike any free-living micro-organisms. Those bacteria and yeasts which spend a part of their life cycle in the lumen of the gut (in Pentatomids, &c., Anobiids, Cerambycids, *Rhodnius*, &c.) are readily cultured outside the body; but with the possible exception of those from Aphids, the intracellular forms have always failed to grow on artificial media. Some doubt, however, exists in this respect about the intracellular bacteria of Blattidae; most authors [84, 99, 110] have been unable to obtain growth in culture; but it is claimed that by using special methods, this organism can become adapted to grow on artificial media, and then appears as a diphtheroid bacillus named *Corynebacterium periplanetae*.[86] But the fact that cultures from newly hatched insects are unsuccessful, raises some doubt as to whether these micro-organisms are indeed the symbiont.[21]

The role of hereditary micro-organisms in nutrition—The constant occurrence of these micro-organisms in a given species, the elaborate mechanisms which ensure their hereditary transmission, and the fact that during development the mycetomes commonly develop in readiness *before* they become invaded, have led to the belief that the organisms are symbionts which contribute something of value in the insect's economy.

This is a subject on which it is impossible to generalize. In origin the organisms have doubtless been parasites; they are sometimes regarded simply as parasites to which the insect has developed an immunity.[143, 163, 185] But it is possible that in the course of evolution they may have become a useful adjunct in metabolism, or even a necessity. Each case requires physiological investigation to decide whether any such benefit to the host exists.[199]

The association of symbionts with the gut suggested that they might be useful in digestion.[34, 181, 186] But there is little support for this view. In the wood-feeding insects there is no relation between the ability to digest cellulose and the occurrence of hereditary symbionts [185]: some Cerambycid larvae have no yeast-containing mycetocytes in the gut and yet are able to digest cellulose [144]; and cellulose is not attacked by yeasts isolated from the gut of those Anobiids and Cerambycids which possess them.[95] Among blood-sucking insects, symbionts are in close relation with the gut in Anoplura, *Rhodnius* and its allies, *Glossina* and Pupipara. In *Glossina* the mycetome lies in the middle of the anterior segment of the mid-gut, where absorption but no digestion takes place (p. 266) (Fig. 227). Digestion begins abruptly where the type of epithelium changes, long after the blood has passed the mycetome.[252] In *Rhodnius*, the bacteria form large colonies in the blood stored in the dilated stomach; but, as we have seen (p. 266), the blood does not suffer digestion until it enters the intestine.[257]

If these organisms are of value it seems more likely that they contribute to nutrition or metabolism. It has often been suggested that they serve to fix atmospheric nitrogen, like the bacteria in the root nodules of leguminous

plants [95]; but there are no quantitative data in support of this. They have been thought to synthesize protein from nitrogenous waste products [115]; for example, in Aphids which lack Malpighian tubes. In the Aphid *Pemphigus*, the reproductive activity of the host and of the symbionts go hand in hand during the year. At the height of viviparous reproduction in *Aphis sambuci*, when the fundatrix is producing offspring equal to her body weight each day, the symbionts are continually growing and undergoing lysis. They are believed to be synthesizing protein for their host.[229]

Another possibility is that they provide accessory factors, and thus enable their host to live permanently on a restricted or highly specialized diet which is incomplete in some respects.[184] It is notable that symbionts occur in practically all the plant-sucking Hemiptera.[34] Among the blood-sucking insects they occur only in those which take no food but blood at all stages of growth; they are absent in mosquitos, Tabanidae, *Stomoxys*, fleas, the larvae of which enjoy a varied diet.[7, 252] We have seen that sterile mammalian blood is an incomplete diet for insects, but that it becomes adequate if infected with bacteria or if extracts of yeast are added to it. The growth of larvae of *Lucilia* on blood is enormously improved if this is infected with the symbiotic bacteria from *Rhodnius*; these seem to furnish vitamins of the B group.[257] In the louse *Pediculus* the symbionts are necessary both for growth and reproduction. Young nymphs deprived of them by centrifuging the eggs always die in about 6 days.[8, 9] In the adult male they degenerate more or less completely; but in the female they are necessary for egg production; and females deprived of them by excision of the mycetome in the last nymphal stage, die in about a week, producing either no eggs or a few non-viable eggs. Partial extirpation produces intermediate effects.[9] All this suggests that the symbionts are contributing a vitamin-like substance; and there is some evidence that yeast extracts may relieve the effects of their loss.[9]

Larvae of the beetle *Sitodrepa panicea* have been obtained free from symbionts by sterilizing the outside of the egg shell (p. 406). Such larvae, reared on 'pea sausage', were still minute at the end of 10 weeks; normal larvae on this diet, and symbiont-free larvae on the same diet plus 25 per cent. of dried yeast, had grown to a large size.[115] On the other hand, *Oryzaephilus surinamensis* which had their symbionts killed by exposure to 36° C. developed normally, and the females gave rise to symbiont-free offspring in which the mycetome developed in the usual way. These grew as rapidly as normal insects and produced an equal number of progeny, even on very poor diets.[115] The species of Cerambycid larvae which have no symbionts develop just as rapidly as those which possess them.[185] Clearly, in these cases the symbionts are not essential to life. But that does not necessarily mean that they serve no useful purpose. It is interesting to note that the persistence of a mycetome without symbionts occurs naturally in certain ants (*Formica rufa*, &c.) [132] and in the weevil *Calandra granaria* [143]; symbionts being still present in *Formica fusca* and *Calandra oryzae*. This again proves that the symbionts are not always essential.

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Chapter XII

Excretion

THE FUNCTION of the excretory system is to maintain a constant internal environment in the body, by the elimination or segregation of unwanted substances present in the blood, and by the retention or reabsorption of constituents needful to the organism. Many organs and tissues contribute to this end, but the most important are the Malpighian tubes, which discharge into the intestine at the junction of the mid-gut with the hind-gut. The urine, the product of the Malpighian tubes, is commonly mixed with the residue of food from the stomach; these together make up the excrement or faeces of insects, the formation and disposal of which have already been discussed (p. 271). But it is possible to study the properties of the urine alone, either by observing the contents of the Malpighian tubes, or by taking advantage of the fact that in many insects there are times when the excrement is made up wholly of the Malpighian secretion.

THE URINE

The role of water in excretion—The character of the urine depends in the first place upon the water relations of the insect. In blood-sucking and plant-sucking forms, immediately after a meal, when there is an abundance of water to be eliminated, the urine is a crystal-clear fluid. Mosquitos will begin passing clear drops of this kind within a few minutes of feeding; the tsetse fly, *Glossina*, will excrete in this way 43 per cent. of its meal in the course of one hour [74]; in two or three hours the blood-sucking bug *Rhodnius*, which takes very large meals, may produce its own weight of urine, and get rid of 75 per cent. of the water in the ingested blood.[134] In aquatic insects, also, the urine is generally copious and clear. Mosquito larvae are continously absorbing water, partly with the food but chiefly by osmosis through the anal papillae (p. 283) and this water is continuously excreted again as urine.[136]

When the water available becomes less, the urine is concentrated. If mosquito larvae are kept in salt water, in which the osmotic uptake of water ceases, the Malpighian tubes are often filled with solid matter (Fig. 242).[136] In the blood-sucking insects, within a few hours after feeding, the urine becomes cloudy; and the sediment increases until the urine attains a creamy consistency. Such insects as *Rhodnius* or the bed-bug *Cimex* live for weeks without ingesting any water, and during this period the urine is in the form of a pultacious mass which dries as a yellow powder. During the pupal stage of insects the whole of excretion takes place without any addition to the water supply. The meconium which accumulates in the rectum, notably in Lepidoptera, and is discharged soon after emergence from the pupa, is com-

posed wholly of urine; and it is of this same type, a heavy, whitish deposit in a yellow fluid.

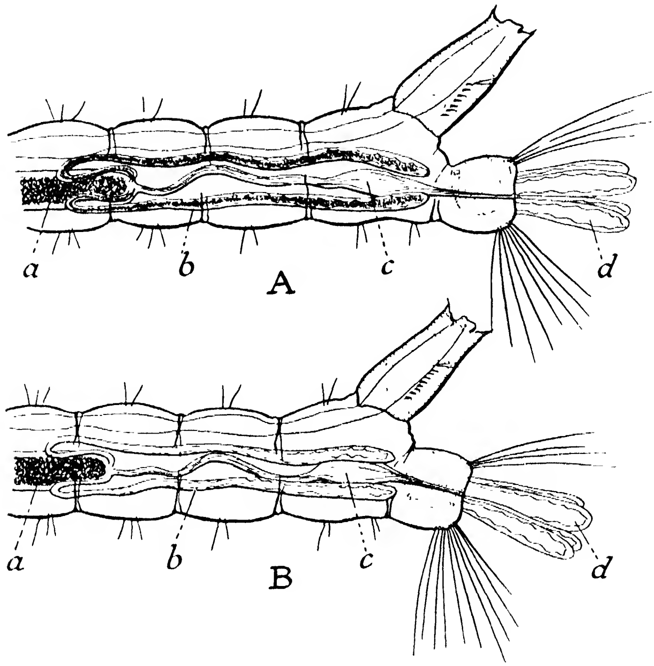

FIG. 242

A, hind end of larva of the mosquito *Aëdes aegypti* reared in salt water, showing solid uric acid in lumen of Malpighian tubes. *B*, the same larva a few minutes after transfer to fresh water; the Malpighian tubes have been flushed out by water entering through the anal papillae. *a*, mid-gut; *b*, Malpighian tubes; *c*, hind-gut; *d*, anal papillae.

We have seen that the excrement of many insects, *Lepisma*, cockroaches, Carabids, caterpillars, &c. is in the form of rather dry pellets from which the water has been extracted by the epithelium of the hind-gut and particularly by the rectal glands (p. 270). In such pellets the urine is present in semi-solid form, sometimes incorporated with the mass, often forming a whitish deposit outside the residue of the peritrophic membrane. And in forms like the mealworm, *Tenebrio*, which live in very dry materials, the urine, like the food residue, is reduced to a bone-dry powder before it is discharged.[32, 135]

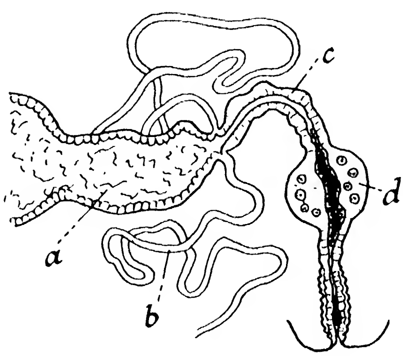

FIG. 243.—A part of the excretory system of *Pediculus*, showing the Malpighian tubes free from solid matter, and solid uric acid separating out in contact with the rectal glands

a, mid-gut; *b*, Malpighian tubes; *c*, hind-gut; *d*, rectal glands.

The urine as it leaves the Malpighian tubes may already contain more or less sediment (in the larvae and pupae of Lepidoptera, pupae of Muscid flies, in mosquitos, *Rhodnius*, &c.); the amount of sediment increases as water is reabsorbed in the hind-gut and rectum. But this reabsorption of water is particularly evident in insects in which the excrement

is solid and yet the Malpighian secretion is perfectly clear. In the human louse, *Pediculus*, which will sometimes produce urine free from faecal admixture, no solid matter appears until the fluid enters the hind-gut. A granular deposit then separates out, accumulates particularly in the region of the rectal glands, and is finally discharged as a moist, white pellet (Fig. 243).[135] In cockroaches, Neuroptera, *Tenebrio*, &c., in spite of the dry state of the excrement, the urine as it is discharged into the gut contains no suspended matter.[135]

Physical characters of the urine—It is obvious that the physical characters of urine which ranges in appearance from a clear fluid to a dry solid, must be extremely variable. The osmotic pressure of the clear urine excreted by *Rhodnius* immediately after feeding is little greater than that of the ingested blood, being equivalent to about 1·0 per cent. of sodium chloride. The fluid accumulating in the rectum at 24 hours after feeding has an osmotic pressure equivalent to 1·7 per cent., and at 48 hours it equals 2·2 per cent. of sodium chloride.[134] No information exists as to the maximum concentration attainable after prolonged fasting in this or other insects.

The reaction must also vary extensively with the diet. The contents of the Malpighian tubes are often stated to be alkaline (in *Galleria*,[83] in *Gnaptor* (Col.)[26, 35]) but in the larvae of *Psychoda* (Dipt.) and *Chironomus* the gut contents change in reaction from alkaline (pH 7·2–7·8) to acid (pH 6·0–6·6) at the point of discharge of the Malpighian tubes[20]; and the meconium of *Deilephila* (Lep.) has a pH of 5·8–6·3, though it is liable to become alkaline as the result of ammonia production through bacterial decomposition.[40] The clear urine of the newly fed *Rhodnius* is alkaline, pH 7·8–8·0, but in the later stages of excretion it gradually becomes more acid until it is about pH 6·0.[134]

The chemical composition of the urine depends upon what substances are present in the diet in excess of the needs of the body, and upon the production of waste products in metabolism. In the nutrition of *Rhodnius* and other blood-sucking insects, the food contains an excess of water and salts, which are rapidly eliminated as a clear solution of bicarbonates and chlorides of sodium and potassium; phosphates of magnesium and calcium appear later and in smaller quantities. Those substances which arise as end products in metabolism do not begin to appear until some hours after feeding. Much of the phosphate doubtless arises in this way, as the end product in the katabolism of materials such as lecithin and nuclein which contain phosphorus in organic combination. Sulphate occurs in small quantities as the end product of sulphur metabolism, coming mostly from the cystine component of the protein molecule. But the most important end product, always present in excess in the proteins of the food, is nitrogen; the elimination of nitrogen is the most important function of the excretory system.

Nitrogenous excretion: ammonia—The simplest form in which nitrogen can be excreted is ammonia. But free ammonia is a toxic substance, and large quantities of water are therefore needed for its elimination; it is seldom important as an excretory substance in terrestrial animals.[24] Ammonia is present, however, in large amounts in the excreta of the meat-eating larvae of *Calliphora* and *Lucilia* during their period of growth,[133] and though a part of this is doubtless the result of bacterial decomposition, much comes from the tissues themselves; for it is formed equally by larvae reared under sterile conditions.[46] But it is not known whether this ammonia is secreted by the

Malpighian tubes or whether it is produced in the alimentary tract (p. 304). When these larvae cease feeding, no more ammonia is produced; and it is absent in the adult fly.[11, 133]

Ammonia combined in salts occurs in the excrement of many insects. In the dried excreta (urine and food residue) of the clothes moth, *Tinea pellionella*, 10·2 per cent. of the nitrogen is present as ammonia, 47·3 per cent. as uric acid [1]; in *Tineola bisselliella* 13·2 per cent. as ammonia, 47·2 per cent. as uric acid [52]; in the grasshopper *Melanoplus* 0·6–0·7 mg. of nitrogen is excreted as ammonia to every 10·6–15·6 mg. eliminated as uric acid [12]; in the meconium of the moth *Antheraea pernyi*, which is composed only of urine, about 0·5 mg. of nitrogen is present as ammonia to 8·7 mg. as uric acid.[19] But it is possible that in some of these cases bacterial decomposition may be in part responsible; for the meconium of *Deilephila* (Lep.) soon comes to contain much ammonia from this cause, whereas when fresh the ammonia nitrogen averages 0·001 per cent., the uric acid nitrogen being about 1·8 per cent.[40]; and the urine of *Rhodnius* does not contain a trace of ammonia at any stage.[134]

Urea, the main nitrogenous waste product of mammals and aquatic vertebrates, is always present in the urine of insects, but in small quantities only; as a trace in the meconium of *Antheraea pernyi* [19, 73]; 0·4 per cent. (uric acid 28 per cent.) in dried excrement of *Tineola*,* [52] 0·3–0·4 per cent. (uric acid 3·2–4·7 per cent.) in the dried excrement of *Melanoplus* [12]; in *Rhodnius* urea is plentiful in the watery urine soon after feeding, and present in traces later.

Of the **amino acids,** leucine has often been claimed as a constituent of insect urine.[111] But the evidence is purely microscopical, soft yellowish spheres present in the lumen of the Malpighian tubes, and remaining after treatment with uric acid solvents, being judged to be crystals of leucine.[120] Chemical analyses have usually shown only a small amount of nitrogen in amino form: 3·1 per cent. (uric acid nitrogen 8·7 per cent.) in the dried meconium of *Antheraea* [19]; 0·35 per cent. (uric acid nitrogen 1·1–1·6 per cent.) in the dried excreta of *Melanoplus*, of which arginine formed a small part [12]; undetermined but small quantities in the urine of *Rhodnius*.

Uric acid is by far the most important nitrogenous constituent in the urine of insects, as it is in birds and reptiles. Uric acid contains less hydrogen than any other nitrogenous compound excreted by animals, and is therefore well adapted for the conservation of water [1]; and being highly insoluble as the free acid or as the ammonium salt, it requires little water for its elimination. These advantages are manifest in free living insects in dry environments; but they are perhaps even more important during the periods of development in the egg or in the pupa, where the insect often has no means of replenishing its water supply.[89]

TABLE 2

NITROGENOUS CONSTITUENTS OF INSECT EXCRETA IN GM. PER CENT.

	Tineola bisselliella (dried excreta) [52]	*Melanoplus bivittatus* (dried excreta) [12]	*Antheraea pernyi* (dried meconium) [19]
Uric acid	28	3·2–4·7	26·2
Ammonia	3·0	0·07–0·08	0·6
Amino-N	—	·35	3·1
Urea.	0·4	0·3 –0·4	trace

* The value of about 8 per cent. given for the dried excrement of *Tinea* must surely be an error.[1]

In the silkworm 85·8 per cent. of the nitrogen is excreted as uric acid [29]; the dried excreta of *Tineola* contain 28 per cent. of uric acid [52]; those of the fasting mealworm *Tenebrio* over 50 per cent. [135]; the meconium of *Antheraea pernyi* contains 5 per cent. of dry matter, 26·2 per cent. of which is uric acid [19, 73]; the meconium of *Deilephila* contains 5·5 per cent. of uric acid in suspension [40]; uric acid, as we have seen, predominates in the excreta of *Melanoplus* (Table 2) [12]; in *Rhodnius*, from 64–84 per cent. of the dried urine is composed of uric acid; this insect, which weighs less than 100 mg., excreting from 0·5–0·6 mg. of uric acid daily during the first week or two after feeding.[134]

When the insect has plenty of water available, the uric acid is in solution (p. 299); when water becomes scarce, it separates out into crystalline spheres. These spherical granules, with a radial striation, ranging in diameter from a fraction of 1μ up to 60μ or more, with an average size of 3–4μ, represent the form in which uric acid and various urates crystallize out of impure solutions (Fig. 244). Their precise composition doubtless varies in different insects.[121] Where much ammonia is present they may contain ammonium urate, as in *Tinea pellionella* [114]; some may contain calcium urate, as in the meconium of *Osmia* (Hym.) [78]; or urates of sodium and potassium. But it is certain that in many cases they consist of free uric acid: in *Rhodnius* 80–90 per cent. of the uric acid is free, the rest presumably as sodium and potassium acid urate.[134] In the meconium of *Deilephila* about half the uric acid is free and half as acid potassium urate.[39] If these spheres are dissolved in very dilute alkali they leave behind a husk or stroma of organic material of unknown composition; if they are immersed in dilute acetic acid or in distilled water, rhombic crystals of pure uric acid separate out.[134]

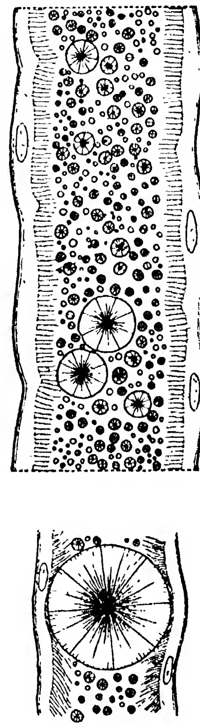

FIG. 244.—Large uric acid spheres in Malpighian tubes of *Rhodnius* starved for several weeks (*after* WIGGLESWORTH)

Other nitrogenous constituents—Guanine, which is important in some other Arthropods, has not been found in insects. Other purines, such as xanthine and hypoxanthine, are absent in the urine of the few insects examined.[73] Allantoine, which arises from the oxidation of uric acid, is present in the excreta of blow-fly maggots.[105] Uric acid accumulates in the tissues of these larvae, they excrete only allantoine; the pupae on the other hand produce much uric acid but no allantoine; the adults excrete both.[13] Allantoine is absent from the meconium of *Antheraea*.[73] Creatine and creatinine are absent in the excreta of *Melanoplus* [12] and the meconium of *Antheraea* [73]; but creatine appears in small quantities in the urine of *Rhodnius*.[134]

Lime and calcium oxalate—The urine of plant-feeding caterpillars is loaded with granules and crystals of many sorts.[110] Besides uric acid and urate granules, calcium carbonate and calcium oxalate are plentiful.[8] The same is true of Phasmids.[120] Amorphous granules of calcium carbonate are produced abundantly, also, by the larvae of many Diptera: they were observed by Lyonnet in *Eristalis* [65]; they occur in Stratiomyids,[129] in *Ptychoptera*,[92] *Calliphora* and

Drosophila,[25] in leaf-mining Agromyzids [124] (in *Acidia heraclei* lime occurs in the Malpighian tubes in the form of 'calcospherites', concentrically laminated granules 8–140μ in diameter (Fig. 245) [58]) and in larvae of *Cerambyx* (Col.).[81]

The significance of the excretion of oxalate is uncertain; possibly it is a method for eliminating oxalic acid present in the food or produced in metabolism. But the lime is probably to be regarded simply as a mechanism for getting rid of the excess of calcium in the food.[66, 92] If there is an excess of fixed base in the food, as is the case with vegetable matter, this excess is most readily neutralized in the urine by the production of alkali urates, or by the retention of carbon dioxide and the precipitation of calcium carbonate. It is noteworthy that although lime occurs in all the leaf-mining Agromyzids, it is absent from the Malpighian tubes of the parasitic Agromyzid *Cryptochaetum*.[124] But, on the other hand, it occurs in the larvae of *Auchmeromyia* (Dipt.) which feed solely on the blood of mammals in which there is only a small excess of base [107]; among Limnobiid larvae it is said to occur more frequently in the predaceous species [61]; and urinary calculi in the rectal caecum of the carnivorous *Dytiscus* have been found to be made up chiefly of calcium oxalate and carbonate.[96] Conversely, where there is an excess of acid-forming matter, as in food rich in sulphur, the urine is most readily neutralized with ammonia. Perhaps that is one factor in the high content of ammonia in the urine of the clothes-moth larvae feeding on keratin, which is rich in cystine.

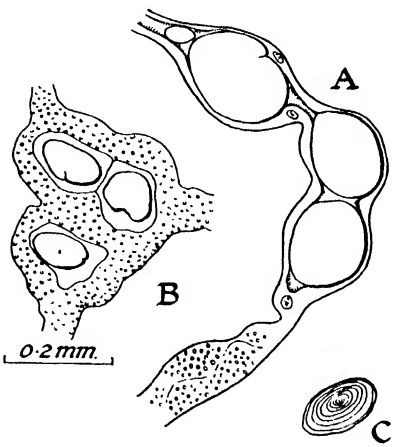

FIG. 245

A, portion of upper segment of Malpighian tubes of larva of *Acidia heraclei* showing calcospherites. *B*, fat body of *Agromyza* larva with calcospherites. *C*, detail of calcospherite showing concentric lamination. (*After* KEILIN.)

Other constituents of the urine—The urine doubtless contains very many complex waste products which are present in such small amounts that they escape detection. There is often a greenish yellow pigment; this has been termed 'entomourochrome' in the belief that it is a single substance and related chemically with the bile pigments of mammals [130]; but this idea lacks chemical proof. The yellow pigment of *Rhodnius* urine seems to be distinct from this.[134] A deep red pigment of unknown chemical nature occurs in the meconium of Vanessid butterflies.[84] The glucoside salicin in the leaves of willows and poplars is generally decomposed in the blood of insects feeding upon these plants and excreted by the Malpighian tubes in the form of salicylic acid.[47]

INTERMEDIARY NITROGEN METABOLISM

Very little is known of the organs concerned in the intermediary metabolism of the nitrogenous waste products, or of the chemistry of their formation. Extracts of blow-fly larvae contain a deaminase with peculiar properties, being water soluble, most active under anaerobic conditions, and capable of liberating ammonia from the larger breakdown products of proteins, such as peptones,

although it is without action upon free amino-acids. The excreta of these larvae do not contain this enzyme, which is presumably intracellular, possibly in the wall of the gut.[14] A deaminase of more usual type, which will liberate ammonia from glycine, is present in the haemolymph of the silkworm.[117]

The chemistry of uric acid formation has been studied only in the larva and pupa of *Antheraea pernyi* (Lep.).[73] Since the purines adenine (6-amino-purine), hypoxanthine (6-oxypurine) and xanthine (2, 6-oxypurine) can all be demonstrated in the larva and pupa, a part of the uric acid (2, 6, 8-oxypurine) is believed to arise by oxidation of these products of nuclein metabolism. But the greater part must arise by synthesis, perhaps by the union of dibasic acids containing three carbon atoms with urea or ammonia. For example, an emulsion of fat body incubated with sodium malonate and urea for 3½ hours at 39° C. gave 84·1 per cent. more uric acid than fat body alone; and this activity was eliminated by boiling. Similar results were obtained with ground-up mid-gut, but not with Malpighian tubes or skin muscles.[73]

Allantoine is produced by the oxidation of uric acid, and the enzyme uricase, effecting this change, has been found very active in the adults of Muscid flies; though absent in *Blatella*, *Melolontha*, *Apis*, and Aphids.[128] Uricase is active also in the pupa of *Antheraea*, all the tissues of which except the Malpighian tubes contain a little allantoine.[73] In *Lucilia*, the pupae of which, as we have seen (p. 303), produce no allantoine, uricase disappears suddenly and entirely when the larvae leave the meat and become 'prepupae'; it reappears abruptly on emergence.[13] Allantoinase, converting allantoine into allantoic acid, has been demonstrated in a long series of adult beetles (Carabids, Dytiscids, &c.) and in *Schistocerca* [106]; but its physiological significance is not known.

MALPIGHIAN TUBES

The Malpighian tubes were so named by Meckel in 1829 after their discoverer Malpighi, who refers to them in his work on the silkworm (1669) as 'vasa varicosa'. Many of the earlier authors such as Cuvier (1802) regarded them as biliary organs. Their excretory function, which was suggested by Herold in 1815, and supported by the finding of uric acid in them by Brugnatelli in the same year, is now generally accepted.[8, 96, 111]

In number they vary from two in various Coccids, to 150 or more in some Orthoptera and in the honey-bee. Where they are numerous they often tend to be short, long when they are few: in *Periplaneta* with 60 tubules they have a total surface area of some 132,000 sq. mm. or 412 sq. mm. per mg. of insect; in the large moth *Gastropacha*, with 6 tubules, they have a surface area of 209,000 sq. mm. or 500 sq. mm. per mg.[111] Where they are few in number they may be several times as long as the insect, but in spite of their great length and twisted course their arrangement is extraordinarily constant in a given species.[121]

The Malpighian tubes may open directly into the mid-gut, often in front of the pyloric sphincter, as in the beetles *Necrophorus* and *Gnaptor* [35]; they open in front of the posterior imaginal ring in Muscids [93]; in the Tettigoniid *Stenopelmatus* they discharge by way of six ureters, which clearly agree with the mid-gut in structure, and are separated from the hind-gut by a valve flap [22];

in Coccids they end far in front of the commencement of the hind-gut.[132] Or they may open equally clearly into the hind-gut, as in Cetoniinae (Col.),[9] and in most caterpillars, where the three tubes on each side unite and discharge into a little chitin-lined bladder.[8, 17]

These facts have led to much controversy as to whether the tubes are ectodermal or endodermal in origin. According to some recent authors, they are believed to arise from the neutral zone where the mid-gut and hind-gut meet [126]; or, more specifically, to arise by ingrowth from the undifferentiated or embryonic cells at the inner end of the proctodoeum, which are perhaps homologous with the lips of the blastopore [43]; their final position being determined by subsequent migration. In the Chrysomelid *Galerucella*, the two short anterior tubes open into the mid-gut, the four posterior tubes are connected by a common chitin-lined stem with the hind-gut.[44]

Anatomical relations of the Malpighian tubes—As a rule the Malpighian tubes lie freely in the body cavity. Occasionally one or more tubes, perhaps

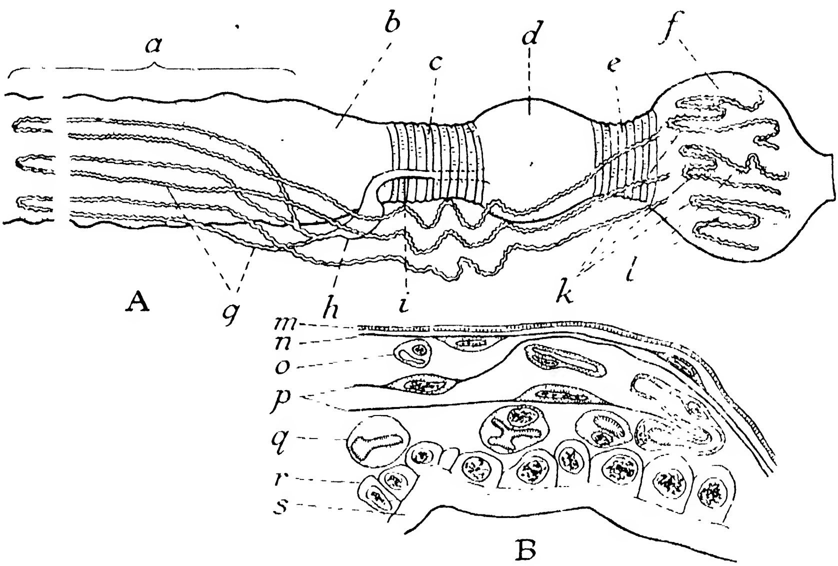

FIG. 246

A, hind part of gut and Malpighian tubes of one side in the larva of *Vanessa urticae*. *a*, mid-gut; *b*, ileum; *c*, anterior sphincter; *d*, colon; *e*, posterior sphincter; *f*, rectum; *g*, free part of Malpighian tubes; *h*, urinary bladder; *i*, free part of common duct; *k*, outer layer of tubes forming the rectal plexus; *l*, inner layer of tubes. *B*, section of rectum and plexus of tubes. *m*, muscular sheet; *n*, outer single membrane; *o*, outer layer of tubes; *p*, inner double membrane; *q*, inner layer of tubes; *r*, rectal epithelium; *s*, lining cuticle of rectum. (*After* HENSON.)

merely as an accident,[104] may enter the ventral ostia of the heart and, leaving by the lateral ostia, come to lie in the dorsal or pericardial sinus.[63] In some insects the terminal portions of the tubes, or some of them, are intimately associated with the wall of the rectum. This arrangement is universal among the larvae of Lepidoptera, with the exception of the Hepialids. The typical arrangement in the silkworm and other caterpillars is as follows. The wall of the rectum is lined by a layer of epithelium covered by thin cuticle; outside this is an inner layer of Malpighian tubes; then, separated by a 'double membrane' made up of two layers of flattened cells, is an outer layer of tubes, the whole being

enclosed in a single outer membrane and a muscular sheath (Fig. 246). The six tubes, the long lower segments of which lie free in the body cavity, enter at the front end of the rectum, run backwards between the single and double membranes, take a convoluted course forwards between the double membrane and the epithelium, and end blindly.[55]

A similar 'cryptonephridial' arrangement of the Malpighian tubes is common among beetles. It takes two main forms: in *Tenebrio*, Chrysomelids, &c., the tubes lie in a single convoluted layer disposed radially around the rectum; in *Anthrenus*,[86] *Gnaptor*,[35] &c., they are united in a tangled mass applied to one side of the hind-gut.[79, 123] In the Mycetophilid larva *Ceraplatus* (Dipt.) the upper parts of the Malpighian tubes, bearing little diverticula, are bound by a membrane to the surface of the posterior half of the hind-gut.[122]

In Myrmeleonid larvae, also, there is a very complex relation between the Malpighian tubes and the intestine.[76] The blind upper parts of the tubes are applied to the hind-gut, and enclosed in a two-layered fold of this consisting of an inner layer of very thin epithelium and an outer of high columnar cells. Between the two is a cavity communicating with the hind-gut.[100]

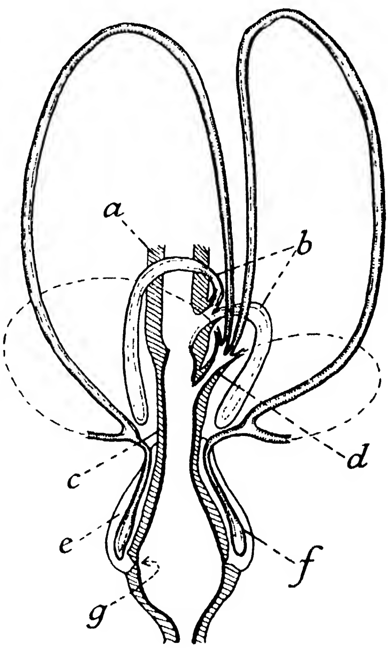

FIG. 247.—Diagram of Malpighian tubes of the Chrysomelid *Galerucella* (*after* HEYMONS and LÜHMANN)

a, mid-gut; **b**, two short Malpighian tubes opening into mid-gut; **c**, hind-gut; **d**, four long Malpighian tubes opening by common chitin-lined duct into hind-gut; **e**, perirectal chamber; **f**, one of the six terminal branches of the Malpighian tubes within the perirectal chamber; **g**, wall of rectum reduced to thin layer of cuticle at this point.

A somewhat different arrangement exists in some Homoptera, where it is the lower end of the Malpighian tubes which comes into relation with the gut. In the Cercopidae, the lower end of each of the four tubes lies between the muscle wall and the epithelium of the 'filter chamber' (p. 267)[75]. In the cicada (*Tibicen septendecim*) the so-called 'internal gland' is a mass of coiled gut and Malpighian tubes, the whole enclosed in muscle fibres.[45]

Sometimes there are obvious anatomical differences between different groups of tubes. In Chrysomelids (*Haltica*,[139] *Galerucella*[44]) the two short anterior tubes, which open into the mid-gut, do not come into intimate relation with the rectum; the four elongated posterior tubes, after a long course in the body cavity, unite on each side into a common stem which then splits up into three terminal branches lying in the chamber on the surface of the rectum. Thus the perirectal chamber contains six terminal segments (Fig. 247). In the Curculionid, *Apion*, there are four long tubes of usual type, and two short tubes forming globular or flask-shaped glands.[53] It is not uncommon for the Malpighian tubes to anastomose with one another to form closed loops, as in *Gnaptor* (Col.),[35] *Tipula*[4] and in Cecidomyids; or even to form crossings through which the lumen is continuous, as in some Homoptera.[75] Sometimes

only a single pair of tubes form closed loops, for example the posterior pair in *Drosophila*.[25]

Before discussing the physiological significance of these anatomical relations we must consider the histological structure of the tubules.

HISTOPHYSIOLOGY OF THE MALPIGHIAN TUBES

The Malpighian tubes are invested by a ' peritoneal coat ' carrying abundant tracheoles and perhaps made up of anastomosing tracheal end cells (p. 184).[26] The tracheal branches bind the tubes to adjacent organs, such as the intestine, which carry them passively to and fro. Or they may be connected to other organs or to the body wall by occasional muscular strands, as in the Tachinid larva, *Thrixion* [91] and in *Ptychoptera* [92]; or a delicate muscle may run from the blind end to the alary muscles of the pericardial septum, as in the anterior tubes of *Drosophila* [25] and *Cryptochaetum* (Dipt.) larvae,[124] so that they are constantly agitated in the body fluids.

Intrinsic muscles—In addition, the tubes of many insects show active twisting movements brought about by a muscular layer beneath the peritoneal coat. These muscles usually consist of a single layer of striated fibres forming wide spirals around the tube. They have been demonstrated, among other insects, in *Hydrophilus*,[71] *Galerucella*,[102] *Oryctes* larva,[68] Gryllids and Locustids,[71, 111] Phasmids,[120] *Periplaneta*, the honey-bee *Apis* (Fig. 248),[88, 127] the dragon-fly *Epitheca*,[16] and in the larvae of Lepidoptera.[8] In other insects such as Cercopids,[75] *Anthrenus* (Col.),[86] *Dytiscus* [108] and the flea (*Ctenocephalus*),[27, 38] the active contortions, twisting and cramp-like movements of the tubes strongly suggest that a muscular coat must exist.

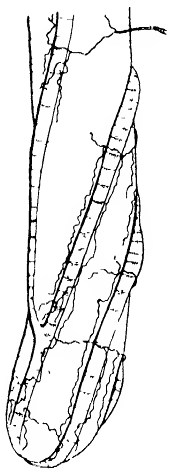

FIG. 248.—End of Malpighian tubule of adult bee, showing tracheoles and spiral muscle fibres (*after* MORISON).

A few muscle fibres, circular and longitudinal, often spread over the lower end of the Malpighian tubes where they enter the gut, so that the terminal duct of the tubes may show an active peristalsis, as in *Drosophila*,[25] Syrphid larvae[65] and *Rhodnius*.[134] Or the thin-walled urinary bladder which marks the lower extremity of the tubes in Gryllidae [7] and the larvae of Lepidoptera,[8] may be invested by delicate longitudinal and oblique muscles. Often this is the only musculature on the tubes: *Hyponomeuta* (Lep.),[54] *Rhodnius*.[134]

Basement membrane and cells—Beneath the muscle coat is a tough elastic homogeneous membrane covering the entire tube. If the tubes of the mosquito are immersed in dilute sodium hydroxide (N/100) the cells swell up and dissolve and are carried down the lumen by an osmotic stream of water, leaving behind this insoluble basement membrane as an unbroken sheath.

The wall of the tube may be made up of a single cell or several; usually one cell will surround half or two-thirds of the lumen. The cells are anchored to the basement membrane by an apparatus which commonly gives an appearance of vertical or radial striation to the basal region of the cytoplasm.[8, 16] The cytoplasm of the cells has most varied characters. It may be diffusely tinged

with green or yellow ; more often it is colourless ; sometimes almost clear, but usually more or less filled with refractile or pigmented concretions or droplets, occasionally with needle-like crystals.[16] The cells are generally discrete with well marked boundaries ; sometimes there may be small diverticula extending from the lumen into the cells, and these caeca may be so large in parts of the tube that this acquires a feathered appearance, as in Melolontha [26] or in Sphingidae (Fig. 249, A).[33] In Cercopidae, the distal varicose segment of the tubes is made up of large cells with intracellular canaliculi communicating with the lumen (Fig. 249, B).[75]

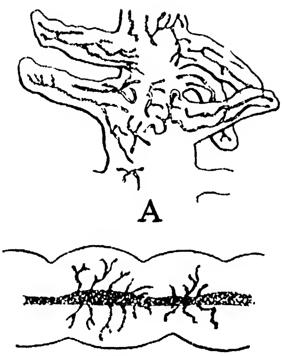

FIG. 249

A, portion of Malpighian tube of *Deilephila*, showing diverticula and highly branched nuclei (*after* GARBARSKAJA). *B*, portion of varicose distal part of Malpighian tube of Cercopid (*cf.* Fig. 229, e) during excretion of indigo-carmine, showing the solid dye in the lumen and in the intracellular canaliculi communicating with it (*after* LICENT).

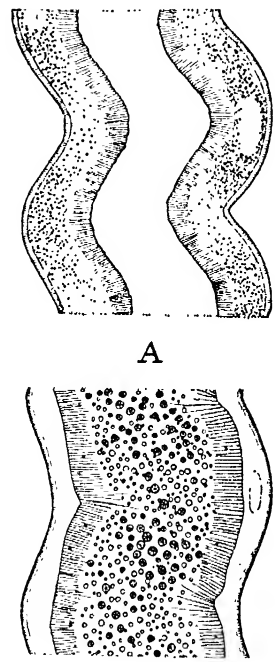

FIG. 250

A, example of Malpighian tube with honeycomb border ; upper segment of tube in *Rhodnius* seen in optical section in fresh material. *B*, example of tube with brush border ; lower segment of same with uric acid spheres in lumen (*after* WIGGLESWORTH.)

The striated border—The inner surface of the cells is covered by a striated border, the structure of which has excited much controversy. It may be accepted at the present time that this border can be of two kinds : (1) the type called ' honey-comb border ' (*Wabensaum*), which appears to be made up of a great number of little rod-like vesicles fused together so as to form a rigid palisade (Fig. 250, A), and (2) the type called ' brush border ' (*Bürstensaum*), which consists of separate filaments quite independent of one another (Fig. 250, B). The distribution of these two types is uncertain because, when the tubes are fixed and sectioned, they are no longer distinguishable with certainty.[134]

The ' brush border ' has been described in the Tenebrionids *Blaps* and *Scaurus*,[72] in the mosquito *Culex*,[70] in *Chironomus* [42] and *Drosophila*,[25] in Gryllidae,[7] Tettigoniidae,[22] Lepidoptera [8] and in *Rhodnius*.[134, 140] The filaments which compose this border are of variable length ; long filaments often overlie the nucleus and the cell boundaries [72] ; and though not actively

motile like true cilia, the longer filaments can be seen waving passively to and fro in the lumen. The reality of the distinction between the two types of border can be seen most convincingly in *Rhodnius*, in which the upper 2·8 cm. of each of the four tubes has a border of the honey-comb type; the border then changes abruptly, and in the lower 1·5 cm. it is of the brush border type.[134] The same distinction probably exists in caterpillars, between the striated border of the perirectal tubes and that of the lower free segments.[54]

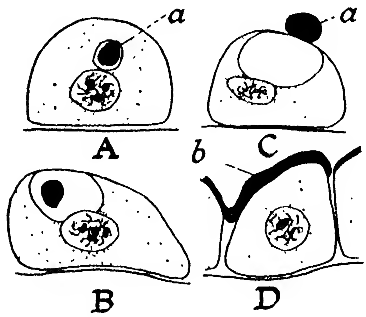

FIG. 251.—Cycle of secretory changes in Malpighian tubules of *Vespa* (*after* GREEN)

a, granule of secretory material being discharged from vacuole; b, accumulated material on the surface of the cells.

Visible changes during secretion—There can be little doubt that the great majority of the changes described as occurring during secretion in the Malpighian tubes are artefacts. The cells of the tubes are exceedingly sensitive to the intense diffusion currents set up by fixatives. Such changes as the distension of the elements of the striated border to form little vesicles,[83, 118, 127] the filling of the cells with vacuoles,[35] the rupture or disappearance of the striated border [83] with the discharge of a foam-like mass of globules into the lumen [33] are certainly due, at least in most cases, to imperfect fixation.[134] The same is true of many of the observations made on isolated tubes in 'physiological solutions'; no artificial fluid is inert towards the Malpighian tubes, which swell up and discharge droplets when so treated.[130, 131] If the tubules of *Rhodnius*, during the most active phases of excretion, are examined, with the minimum of injury, in the insect's own blood, they show only a general distension of the lumen and, in the region of the brush border, the passive moments of the elongated filaments mentioned above.[134]

On the other hand there may be more than one mechanism of excretion.[26] In *Vespa* a secretory granule staining darkly with haematoxylin appears internal to the nucleus, enlarges as it is moved inwards, and is discharged into the lumen to fuse with other granules and so to form a thick layer over the cell surfaces (Fig. 251).[37] A similar process is described in *Apion* (Col.) [53] and *Dytiscus* [108]; and in some other insects portions of the cells containing solid deposits of excretory substances are said to be pinched off and set free in the lumen.[16]

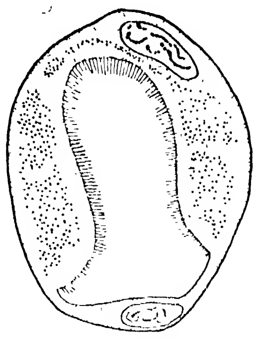
FIG. 252.—Transverse section through Malpighian tubule of *Ptychoptera* larva showing the two types of cell of which it is composed (*after* PANTEL)

The formation of urate granules—In most insects the granules present in the cytoplasm of the Malpighian tubes are not the same as the excretory granules in the lumen.[109, 130] The refractile spheres in the cells in mosquitos, which appear superficially very like uric acid, are not blackened by ammoniacal silver nitrate like the granules of uric acid in the lumen.[138] The secretion of the cells is in fact generally fluid; the concretions and crystals separate out later. Precipitation is probably induced by a reabsorption of water by the cells.[16] We

have seen that this certainly happens in the hind-gut and rectum ; but in many insects, such as Muscid flies during pupal life, and the adults of mosquitos, the tubes themselves contain heavy deposits of uric acid throughout their length. In such insects it is not known whether the cells pass through alternate phases of secretion and reabsorption, or whether different cells perform these two functions constantly. But in some insects a dimorphism has been observed in the cells of the tubes ; in the adult *Ptychoptera*, among the pigmented cells there are scattered thin cells devoid of pigment and without a striated border (Fig. 252) [92] ; similar interstitial cells devoid of granules occur in *Anopheles*,[85]

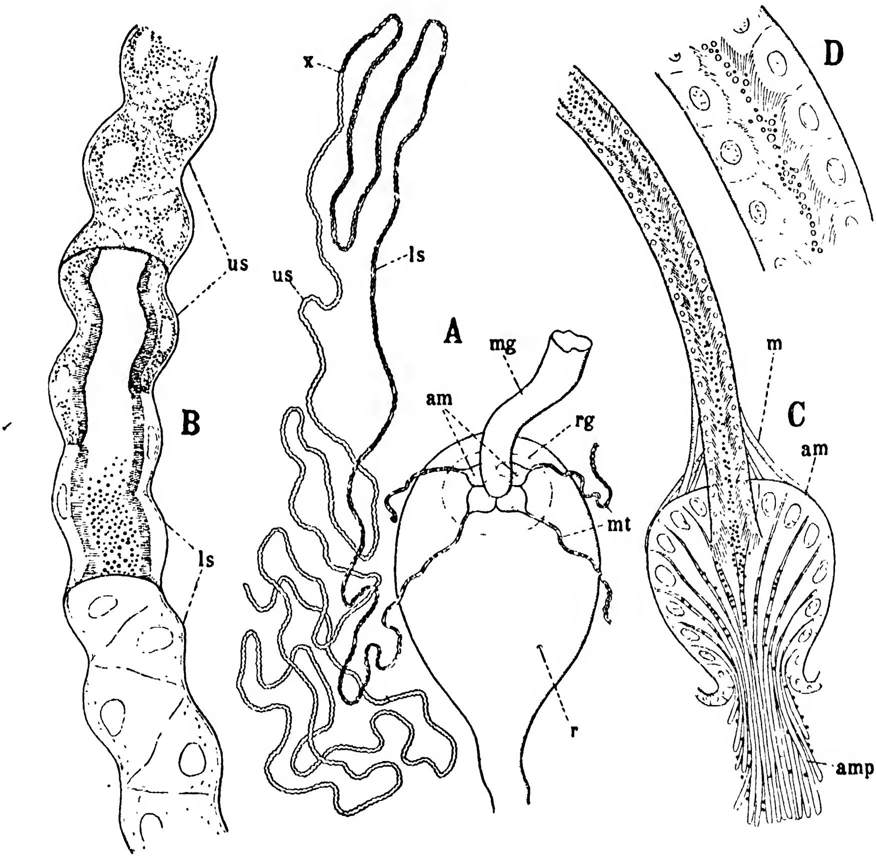

FIG. 253

A, excretory system of *Rhodnius* ; only one Malpighian tube shown in full ; *B*, junction of upper and lower segments of Malpighian tube, seen in surface view and optical section ; *C*, lower end of Malpighian tube and terminal ampulla seen in optical section ; *D*, detail of this region of tube (*after* WIGGLESWORTH). *am*, ampulla ; *amp*, processes of ampulla cells ; *ls*, lower (proximal) segment of Malpighian tube ; *m*, muscle fibres ; *mg*, mid-gut ; *mt*, Malpighian tubes ; *r*, rectum ; *rg*, rectal gland ; *us*, upper (distal) segment of Malpighian tube ; *x*, junction of upper and lower segments of tube.

in the larva of *Galleria*,[83] and in the beetle *Dromius*.[111] These cells may possibly be concerned in reabsorption.

A more definite division of labour is seen in *Rhodnius*, in which the granules of uric acid occur only in the lower third of each tube where the cells bear a brush border ; the upper two thirds of each tube with the honey-comb border containing only clear fluid (Fig. 253). In *Rhodnius* there seems little doubt that the upper segment is concerned only with secretion, the lower segment with

reabsorption. For if the very diffusible dye neutral red is added to the blood of the insect, it can be seen quite clearly to pass through the cells into the lumen in the upper segment, and to be absorbed from the lumen into the cells in the lower segment. Moreover, if two ligatures are applied to the lower segment of the tube some hours after feeding (at a time when all uric acid granules have

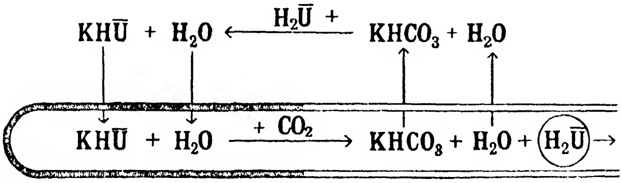

FIG. 254.—Diagram showing possible mechanism by which free uric acid is precipitated in the lower segment of the Malpighian tubes in *Rhodnius* (*after* WIGGLESWORTH)

Shaded part represents upper segment of tube.

been swept away by the rapid flow of urine) uric acid will separate out above the upper ligature, but between the ligatures no uric acid appears, nor is there any distension of this part of the tube, as would happen if secretion were taking place.

In *Rhodnius*, and this may apply to other insects also, there seems to be another mechanism which favours the precipitation of uric acid. The contents

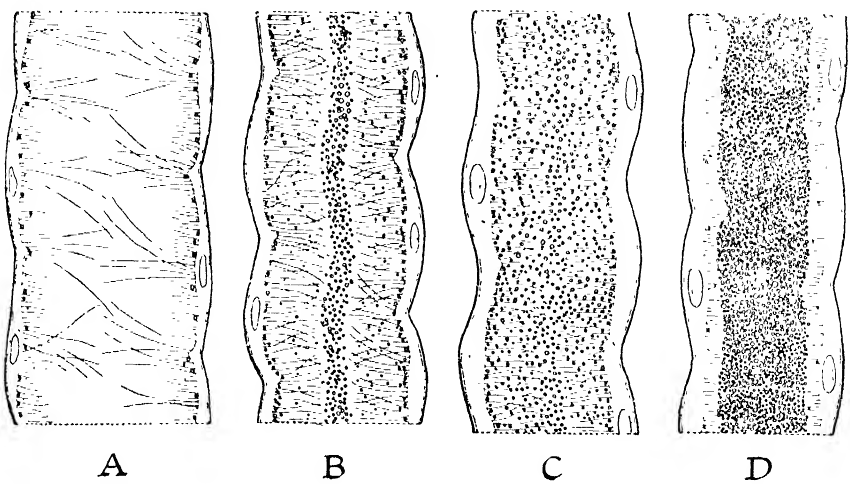

FIG. 255.—Optical sections of lower segment of Malpighian tubes in *Rhodnius* showing variations in the state of the filaments (*after* WIGGLESWORTH)

A, 4 hours after feeding ; lumen is distended, many of the filaments are very long, uric acid granules beginning to appear around bases of filaments. *B*, 12 hours after feeding ; all filaments elongated, some uric acid granules between filaments, others free in a central channel. *C*, 24 hours after feeding ; all uric acid granules entangled among elongated filaments. *D*, 3 days after feeding ; filaments contracted, almost all uric acid spheres free in lumen, a few between the filaments.

of the upper segment of each tube are faintly alkaline (*p*H 7·2), in the lower segment they are acid (*p*H 6·6). This suggests that the uric acid is secreted in the form of the relatively soluble potassium or sodium acid urate in the upper segment, and that the base is reabsorbed along with the water in the lower segment of the tube, leading to the precipitation of free uric acid (p. 303).

Thus the same water and base are circulated and used repeatedly in excretion (Fig. 254).

During this process of reabsorption in the lower segment, the filaments of the brush border may become so elongated as to fill the entire lumen, and hold the uratic spheres among them as in a gelatinous mass. Later, the filaments retract, and the spheres are carried down the free path in the lumen (Fig. 255). Further reabsorption takes place in the peculiar ampullae at the lower ends of the tubes, and in the rectum.[134]

Specialization of parts of the Malpighian system are common in other insects.[109, 110] In Gryllidae some tubes are yellow, others white and loaded with uratic concretions [60]; and in *Gryllotalpa* the dye indigo-carmine is excreted only by the yellow tubes.[21, 62] In nymphs of *Heptagenia* (Ephem.) there are more than 100 tubes, each made up of three very distinct segments.[80] In the larva of *Dytiscus*, the proximal parts of the four tubes differ histologically from the distal.[3] In the imago of *Borkhausenia pseudospretella* (Lep.) as in *Rhodnius*, uratic spheres occur only in the lower segments of the tubes.[135]

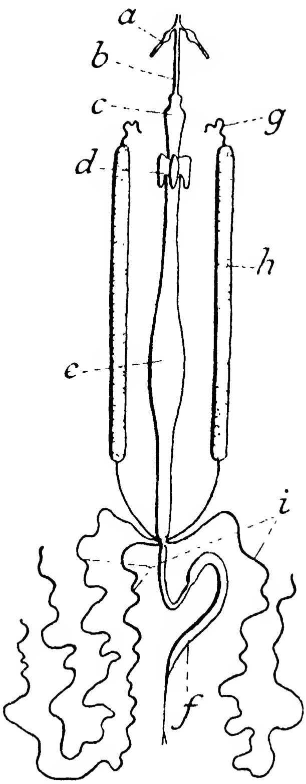

Fig. 256.—Alimentary canal and Malpighian tubes of larva of *Ptychoptera*; schematic (*from* Pantel *after* van Gehuchten)

a, salivary glands; b, fore-gut; c, proventriculus; d, caeca of mid-gut; e, mid-gut; f, hind-gut; g, extremity of anterior Malpighian tubules, of normal appearance; h, middle segment of anterior tubules transformed into a lime sac; i, three posterior tubules of normal structure.

Formation of lime granules—Lime usually appears in the Malpighian tubes as a milky suspension of amorphous granules up to 3·5μ in diameter, which dissolve in acids, leaving behind only a minute particle of some organic material [58, 92]; occasionally the lime forms 'calcospherites' (p. 304). The granules are formed in the lumen of the tubes; but nothing is known of the mechanism of their formation. They are generally confined to some specific part of the system: the distal half of one pair of the four tubes in Syrphids and Stratiomyids [65, 129]; in the distal halves of the anterior pair of tubes in the larvae of *Drosophila* and *Calliphora* and other Diptera [25]; in the dilated tips of the corresponding tubes in the parasitic *Thrixion* [91]; in the dilated middle region of the anterior pair of the five tubes in *Ptychoptera* larvae (Fig. 256) [92]; in certain restricted regions of the posterior group of tubes of many female Phasmids [120]; and in the four long tubes of the larva of *Cerambyx* (Col.), not in the two short tubes.[81] The segments where they occur are usually greatly distended, the cells attenuated, with no vestige of a striated border (Fig. 245, A).[92]

The significance of the cryptonephridial tubes—We have seen that in the larvae of Lepidoptera, in many Coleoptera and in the ant lions, the Malpighian tubes come into intimate relation with the hind-gut (p. 306). In caterpillars, these upper segments of the tubes are so completely cut off from

the body cavity that they cannot take up the general waste products; dyes injected into the body cavity are taken up only by the free segments of the tubes.[83] We have seen that the tubes surround that segment of the hind-gut in which water is extracted. It has been suggested that toxic substances of unknown nature may be absorbed at the same time, and that the function of these parts of the Malpighian tubes is to remove all harmful substances before the fluid passes into the body cavity.[83] But it is possible that the water absorbing power of the gut wall is increased by having another water absorbing mechanism outside it; and that the perirectal tubes are simply part of the mechanism for recovering water from the excreta; this water being returned to the body cavity by the lower segments of the tubes, or used to flush out the solid matter from the lumen.[101] The tubes of the convoluted inner layer have a much thicker epithelium than in the outer layer; perhaps the function of the two layers is different.[55]

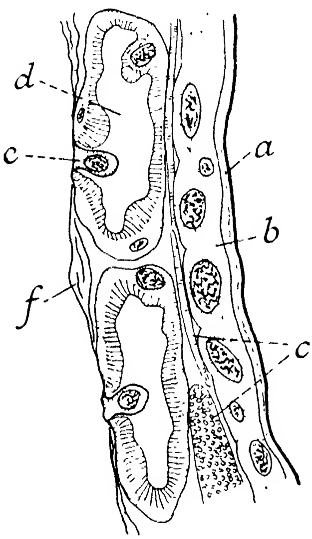

FIG. 257.—Section through the wall of the rectum in the adult of *Tenebrio molitor* (*after* CONET)

a, cuticular lining of rectum; *b*, rectal epithelium; *c*, muscles; *d*, lumen of Malpighian tube; *e*, specialized cell separating the lumen from general body cavity; *f*, peritoneal sheath.

In beetles, the cryptonephridial arrangement occurs in phytophagous, carnivorous and omnivorous species.[79] Fluid is certainly taken up from the gut contents and conveyed to the granular blood-filled space in which the tubes lie. Sometimes (in *Tenebrio*, &c.[18]) the gut is here covered with large absorbing cells; sometimes its contents are separated from the Malpighian tubes only by a thin-walled window (as in *Anthrenus*[86]); or there may be several points at which the wall is reduced to a thin membrane (as in Chrysomelids[44, 139]). The chamber in which the tubes lie is bounded externally by a 'perinephric membrane', and at the points where the convoluted underlying tubes come in contact with this, the wall of the tube is attenuated so as to form a great number of pores closed only by a very thin membrane (in *Adalia*, *Agelastica*, *Gastroidea*, *Melasoma*) or occupied by a cell with large vesicular nucleus, which is clearly differentiated from the other cells that make up the wall of the tube (in *Blaps*, *Bruchus*, *Tenebrio*) (Fig. 257).[18, 99]

These segments of the tubes almost certainly take up water reabsorbed from the rectum. They have been regarded as helping in the conservation of water (as in Scolytids,[115] Tenebrionids,[98] Chrysomelids[44]); an idea supported by the fact that cryptonephridism does not occur in the aquatic beetles, not even in Chrysomelids like *Donacia*, although this arrangement exists in all other members of the family.[98] And they have been supposed, as in Lepidoptera, to purify the absorbed fluid before it is returned to the body cavity.[35, 44, 98, 139] But some doubt exists as to whether fluid or other substances pass through the thin walled pores from the lumen of the tubes to the body cavity, or in the reverse direction.[18] Most observers have found that indigo-carmine and other dyes, injected into the body cavity, are taken up only by the free portions of the tubes[35, 44]; but in *Tenebrio* they are said sometimes to enter the perirectal segments.[99]

In Myrmeleonid larvae, also, the arrangement is regarded as being concerned with reabsorption from the hind-gut. The curious fold of the hind-gut which invests the upper parts of the Malpighian tubes is perhaps homologous with the rectal glands of the adult, which almost certainly absorb water. Fluids absorbed by the hind-gut, enter the perinephric space; part is then believed to be secreted into the lumen of the Malpighian tubes, part to be taken up by the high columnar cells of the investing fold and returned to the haemocele.[100] Injected dyes may reach the upper segments of the tubes by this route.

ACCESSORY FUNCTIONS OF MALPIGHIAN TUBES

The Malpighian tubes are sometimes regarded as regions of the intestine in which the primitive excretory functions of the gut have become centred.[130] We have discussed elsewhere the extent to which they contribute to digestion (p. 276); here we shall consider new functions they may occasionally acquire. A sticky secretion, provided apparently by modified cells in the Malpighian tubes, is poured out upon the terminal pseudopod (*Nachschieber*) of certain Chrysomelids, notably *Agelastica alni*, and serves to aid progression.[10] In other Chrysomelids the Malpighian tubes furnish the sticky substance with which the female covers her egg chambers.[44] The viscous principle, possibly related to silk, which makes the foam sheltering Cercopid nymphs permanent and resistant, is believed to come from the Malpighian tubes, being mixed with fluid from the gut and whipped into a foam by the repeated closure and separation of the terminal sclerites. The middle segment of the tubes in these nymphs, which is dilated and has branched nuclei, is thought to be the source of this secretion.[75]

Undoubted silk is produced by the Malpighian tubes in several groups. In the larva of *Chrysopa* (Neuropt.), after the 2nd instar, a striking difference appears between the fore-part and hind-part of the tubes. The hind-part is of normal character; in the fore-part the cells become greatly thickened, increasingly so towards the time of spinning the cocoon; the nuclei, round before, become enormously branched and the lumen filled with viscid secretion. There is a sharp transition between these two zones. In the imago, which no longer produces silk, the tubes have the same character throughout their length as the hind-part of the larval tubes.[77] In Myrmeleonid larvae, also, the lower parts of the tubes are concerned in silk secretion, and show similar changes[76]; and the rectal sac serves as a reservoir for silk at the time of pupation.[100] In the larvae of *Phytonomus* (Col.), the Malpighian tubes are massively developed, and are responsible for producing both the silk tissue of the cocoon (from the middle segment of the tubes) and the coating materials which are smeared over its lining (from the larger apical region).[69] Other beetles in which the Malpighian tubes secrete silk are *Lebia scapularis*[119] and *Niptus hololeucus*.[79]

In the cocoons of Lepidoptera the silken tissue is produced by the labial glands, but the smeared coating in *Leucoma salicis*, *Eriogaster lanestris*, &c., is provided by the excreta[23]; the lemon yellow powder used for this purpose by *Malacosoma neustria* consists of urates from the Malpighian tubes.[34]

Finally, the lime collected in the tubes is often utilized. The Australian Cercopid *Ptyelus*, living on Eucalyptus trees, forms a spiral shell consisting of at least 75 per cent. calcium carbonate.[103] The lime in the tubes of *Cerambyx*

larvae passes to the stomach and is ejected through the mouth to form an operculum for the burrow.[81] The great calcospherites in the tubes of *Acidia heraclei*, which are too large to pass down the lumen, dissolve in the blood during the first day of metamorphosis, pass through the newly formed cuticle of the pupa, and are deposited as a hard and brittle layer on the inner surface of the puparium.[58] A similar transference of lime from the Malpighian tubes to calcareous pits in the cuticle takes place at each moult in the larvae of Stratiomyids.[66] And in female Phasmids, lime stored in the tubes is reabsorbed into the blood and incorporated in the chorion of the eggs.[120]

MALPIGHIAN TUBES DURING MOULTING AND METAMORPHOSIS

The Malpighian tubes of caterpillars show a cycle of changes during moulting. Shortly before the skin is cast, there is evidently a copious secretion of fluid by the tubes, which carries down the accumulated crystals of urates and oxalates. These pass between the old cuticle and the new in the ureters and rectum, gush out around the margins of the anus, and spread forwards over the body beneath the old cuticle.[116] They become mixed with the moulting fluid and may be swallowed by the mouth.[36] After moulting the tubes are almost devoid of crystals.

The changes undergone by the Malpighian tubes at metamorphosis differ in different groups. In the Diptera and Coleoptera they suffer little change.[56, 93, 102] In those forms which contain lime, this is generally emptied into the gut at the time of pupation,[81, 92] and the tubes assume the same histological character throughout. In *Ptychoptera* the lime-containing segment is sealed below by a syncytial fusion of cells across the lumen; this obstruction disappears at pupation.[92] In Lepidoptera, the perirectal segments of the tubes break down and are removed by phagocytes [17, 54]; the free segments persist and increase in length, and their epithelium is reconstituted to form the adult tubes. The interstitial ring of embryonic cells at the base of the tubes seems to contribute little to their formation.[43] In Hymenoptera, the four Malpighian tubes of the larva break down completely during histolysis (as in the ant [57, 93] and the bee [6]). The adult organs are a new formation which arise as numerous short finger-like outgrowths from the annular swelling at the junction of the mid-gut and the hind-gut.[6] In the Chalcid *Nasonia*, Malpighian tubes develop for the first time in the pupa.[125]

CEPHALIC EXCRETORY ORGANS AND INTESTINAL EXCRETION

'Labial Kidneys'—Malpighian tubes are absent, probably through reduction, in Collembola, in *Japyx* among Thysanura, and in Aphids. Little is known of the excretory organs of Aphids, but in Collembola and Thysanura there are several pairs of glands in the head. Of these, the 'tubular glands', opening by a common duct above the base of the labium, are regarded as excretory organs homologous with the cephalic nephridia of other groups.[15, 95a]

In general arrangement the tubular glands are very like the antennal glands of Crustacea. They consist of an upper region or 'saccule' with flattened

epithelium, communicating with a long coiled tube or 'labyrinth', the cells of which resemble those of the Malpighian tubes.[15] A third gland discharges into the duct of the tubular gland (Fig. 258). These structures have substantially the same form in all the Apterygota. Nothing is known of the normal products of their excretion; but the saccule takes up ammonia carmine injected into the body cavity; and the labyrinth takes up indigo-carmine, passes it through the cells in the reduced or colourless form, and deposits it in the form of blue crystals in the lumen, just like the Malpighian tubes.[15] In this these organs resemble the 'green gland' in Crustacea.[62]

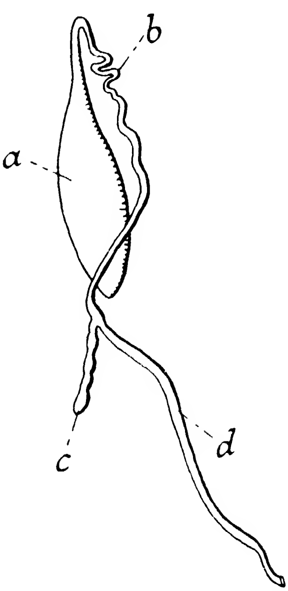

FIG. 258.—Tubular gland of Collembola (*after* MUKERJI) a, saccule; b, labyrinth; c, dorsal loop; d, duct.

Excretion by the gut—There is no satisfactory proof that the gut wall retains in insects the excretory functions it has in many groups.[26] During metamorphosis, notably in Hymenoptera, quantities of uric acid granules may appear in the mid-gut. This has led to the belief that the mid-gut epithelium may be important in excretion, for instance in Sphegidae.[28, 78, 130] But in many, if not all of these cases, the uric acid has passed forwards from the Malpighian tubes. In Collembola the epithelium of the mid-gut is periodically cast off, and this has been regarded as an excretory process [31]; and uric acid has been demonstrated in the mid-gut of the beetles *Gnaptor* and *Necrophorus*,[35] though it is absent in the silkworm.[67]

STORAGE EXCRETION

Urate cells—In the Collembola, which lack Malpighian tubes, granules of uric acid collect in certain cells, the so-called 'urate cells', scattered through the substance of the fat body; these cells become increasingly loaded with concretions as the insect grows older.[95a] And in the cockroach, the Malpighian tubes, though present, are said never to contain any uric acid [21]; this accumulates throughout life in discrete urate cells which appear in the developing fat body of the embryo [30] and increase in volume, and in the size of the concretions they contain, throughout the life of the insect (Fig. 259).[21, 95]

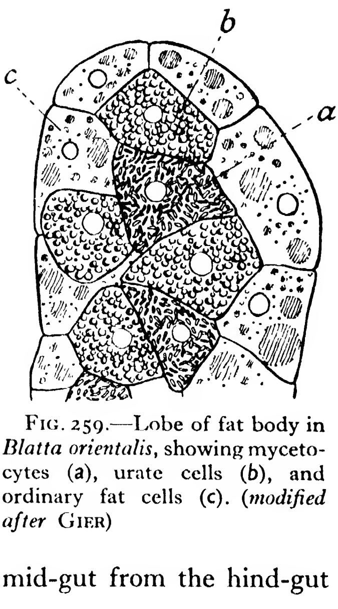

FIG. 259.—Lobe of fat body in *Blatta orientalis*, showing mycetocytes (a), urate cells (b), and ordinary fat cells (c). (*modified after* GIER)

In the larvae of Hymenoptera,[93] the urate cells appear as white points dotted among the cells of the fat body. During the larval stage of some species, such as the honey-bee, *Apis*, the Malpighian tubes end blindly below in the substance of the diaphragm which separates the mid-gut from the hind-gut (p. 256). As the larva grows the tubes become

distended, and their walls very thin, but they contain no uric acid; only towards the time of pupation do they establish their connexion with the hind-gut [90]; and only during pupal life, or soon after the emergence of the adult, is the uric acid accumulated in the urate cells transferred to them and voided into the gut.[78, 135] In the Chalcid *Nasonia*, Malpighian tubes are entirely wanting in the larva.[125]

In these examples the storage of uric acid within the tissue cells clearly replaces, temporarily or permanently, its elimination by the Malpighian tubes. But there are other insects in which uric acid may crystallize out in the tissues even when the Malpighian tubes are functional. It may appear in the ordinary fat body cells of *Culex*, larvae and adults,[5] of *Dytiscus* [64] and other Coleoptera,[35] and of the larvae of Lepidoptera; or in the epidermal cells of many caterpillars, such as the silkworm,[121, 28] and various Tenthredinids; in certain restricted

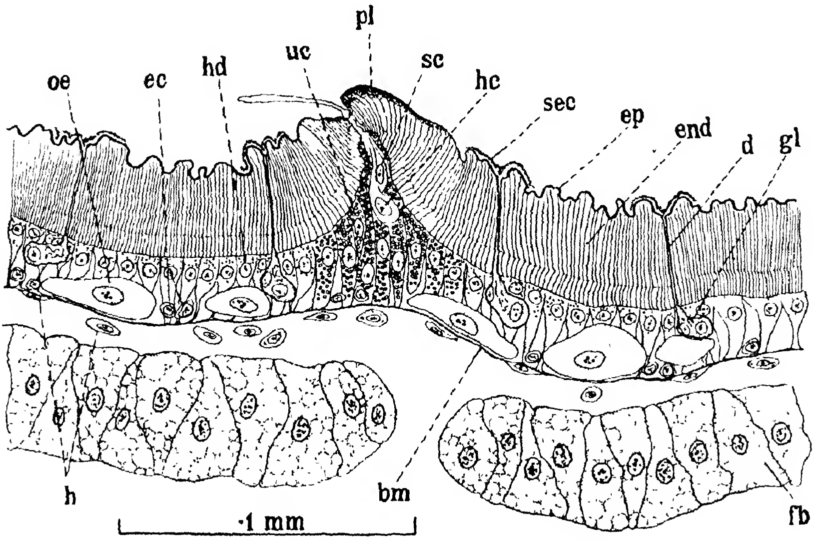

FIG. 260.—Section through the integument of nymph of *Rhodnius* to show the underlying tissues (*after* WIGGLESWORTH)

bm, basement membrane; *d*, duct of dermal gland; *ec*, embryonic cells; *end*, endocuticle; *ep*, epicuticle; *fb*, fat body; *gl*, dermal gland; *h*, haemocytes; *hc*, hair-forming cell; *hd*, epidermal cells; *oe*, oenocytes; *pl*, plaque, bearing bristle; *sc*, socket-forming cell; *sec*, dried secretion from dermal glands; *uc*, cells stuffed with spheres of uric acid.

parts of the epidermis of aquatic Hemiptera (*Notonecta*, &c.) during their nymphal stages,[97] and in the nymphs of *Rhodnius* during moulting (Fig. 260).[137] In such cases it seems reasonable to suppose that the uric acid has been produced during the metabolism of the cells in question, and has merely been caused to crystallize out by the conditions, acidity and so forth, existing within them.

That raises the question whether, in general, uric acid is produced within the urate cells, or collected by them from the blood. In *Vanessa* larvae (Lep.), urates first appear in the fat body, as minute refractile granules around the nucleus, towards the time of pupation. If ammonium urate is injected at this time, urates are excreted by the Malpighian tubes, but the granules in the fat body show no increase.[49] Similar negative results have been obtained with the urate cells of Locustids [51] and Blattids,[95] though in the cockroach the con-

cretions do increase after prolonged starvation, presumably as the result of the utilization of stored protein.[95]

These observations suggest that the uric acid is formed within the cells themselves. That certainly appears to be the case, also, in Muscid larvae, in which, towards the time of pupation, darkly staining ' pseudonuclei ' appear within the albuminoid inclusions laid down in the fat body (p. 240) [2] and become transformed into concretions of uric acid (Fig. 209).[93] During the last few days of pupal life these ' pseudonuclei ' disappear from the inclusions ; the uric acid set free is taken up by the Malpighian tubes, and accumulates as meconium in the rectal pouch.[93, 135] A similar transference of urates from fat body to meconium occurs, as we have seen, in Hymenoptera [78] ; and from the fat body and epidermis, in Lepidoptera.[21, 28, 36] Thus, although the uric acid deposited in the cells of the tissues is probably the product of their own metabolism, it often represents a true storage excretion, tiding the insect over the period when the Malpighian tubes are being reconstituted.[94]

In those insects in which the urate cells are distinct from the ordinary cells of the fat body, as in *Lepisma*, Collembola, and the cockroach, &c., they probably arise in the course of development from fat cells.[95] But in the young larva of the ant [93] and bee [113] they can be recognized as distinct rounded cells, without concretions, which later become applied to the surface of the fat lobes. In the Tenthredinidae they are completely buried in the fat cells,[93] and have been described as arising from leucocytes which penetrate these cells during larval life.[112]

Other examples of storage excretion—There are many other waste substances besides uric acid which occasionally accumulate in the tissues. Lime in the form of ' calcospherites ' is common in special cells associated with the fat body in the larvae of phytophagous Diptera (Fig. 245).[58] In larvae of *Phytomyza*, mining the leaves of Cineraria, there may be anything up to 60 of these intra-cellular pellets of lime, each exceeding 200μ in length. During histolysis in the pupa, they are set free and dissolve ; they are absent in the adult.[41] The fat body, and special tracts of the epidermis, in old termites become filled with yellow brown granules, perhaps of some waste product.[87] When the larvae of *Tortrix viridana* (Lep.) and other insects feed on the leaves of oak, crystals, believed to be derived from tannin, appear in the cells of the gut wall.[50] The fat body of *Cionus olens* (Col.), feeding on flowers of *Verbascum*, contain granules of purple pigment apparently identical with the anthocyanin in the stamens.[48]

But it is the pericardial cells and the other so-called nephrocytes (p. 237) which are most apt to accumulate waste substances. As we have seen, the readiness with which ammonia carmine and certain other dyes collect in these cells gave rise to the belief that their main function was the segregation of waste products (p. 238).[62] And although that view is probably mistaken, deposits of various kinds may occur naturally in these cells. The brownish granules common in old termites are very abundant in the pericardial cells.[87] The green pigment in these cells in Hemiptera and the carotinoids in some other groups are perhaps excretory.[49a] The pericardial cells and the ventral nephrocytes of *Lonchaea chorea* (Dipt. Acalypt.) feeding on decomposed mangel-wurzels, become so laden with brown granules of pigment that they may break down and be consumed by phagocytes.[59]

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Chapter XIII

Metabolism

METABOLISM COMPRISES all the chemical changes which the constituents of the living body undergo. Broadly speaking, these changes serve two purposes, growth on the one hand and the performance of work on the other. They may be studied by following the chemical transformations or changes in composition of the body, by observing the energy changes, particularly the production of heat, that accompany the chemical phenomena, or by following the consumption of oxygen and the evolution of carbon dioxide, which give an indirect measure of the energy changes that are taking place.

CHEMICAL TRANSFORMATIONS

Fat metabolism—Fat is the chief form in which energy is stored. It is usually present in greatest amounts in the mature larva before metamorphosis. In *Gastrophilus* it rises from 3·5 per cent. of the dry weight in December to 25·9 per cent. in June.[103] In the mature larva of *Calliphora* fat comprises 22 per cent. of the dry weight,[196] of the corpse-fly *Ophyra cadaverina* 41–45 per cent.,[173] of the honey-bee worker 18 per cent., of the drone 21·3 per cent.,[171] of *Bombyx mori* 7·1 per cent.,[55] and of *Galleria* 43·6 per cent.[164]

In composition this fat is exceedingly varied. The unsaponifiable fraction is usually small (1·5–1·6 per cent. in the 'chrysalis oil' of *B. mori*,[11] 1·56 per cent. in the mealworm larva [10]). In the 'chrysalis oil' a large part of this fraction is composed of hydrocarbons; about one third consists of sterols, of which 85 per cent. is cholesterol, 15 per cent. sitosterol.[11] By far the greater part of the 'total fat' or 'ether extract' consists of neutral fats. These usually contain a rather high proportion of unsaturated acids. In the mealworm, for example, the fatty acids are: palmitic and other saturated acids 22·6 per cent., oleic 44·7 per cent., linoleic 32·3 per cent., linolenic 0·35 per cent. [10]; and in 'chrysalis oil', palmitic 20 per cent., stearic 4 per cent., oleic 35 per cent., linoleic 12 per cent., linolenic 28 per cent.[11] Such high values for linoleic and linolenic acids are quite unlike those found in mammals. Thus the iodine value, which affords a measure of the degree of unsaturation, is generally high: 117 in silkworm oil, 112–159 in other Lepidopterous larvae, 108·6–118 in the phytophagous Chrysomelids, 68·5 in the xylophagous beetle *Ergates faber*, but ranging as low as 37·3 in *Gastrophilus*.[184]

At ordinary temperatures the fat may be a liquid oil, as in *Ergates faber*, or a crystalline solid, as in *Oryctes nasicornis*, both of these being wood-feeding beetles.[184] In the eggs of grasshoppers it seems to be a general rule that those which pass the winter in this stage have fats of lower melting point: in *Melanoplus differentialis*, which spends the winter as an egg, the fat is liquid at room

temperature; in *Chortophaga viridifasciata*, which winters as a nymph, the fat is solid. In this case the iodine number is the same in both (135–140) [168]; but the low melting point in the winter eggs may possibly be due to a higher proportion of short chain fatty acids. That is the explanation of the liquid fat of the Aphid *Pemphigus*, which contains glycerides of butyric, caprylic and lauric acids.[184]

The neutral fats are doubtless formed in great part from carbohydrates or proteins in the food. *B. mori* at pupation contains twice as much fat as in the leaves it has eaten [127, 189]; and fly larvae will lay down fat when fed on fat-free albumen.[136] When the food is rich in fats, the fat laid down by the insect is influenced by their composition, but it is sufficiently different to prove that the fat is extensively changed in metabolism: there is not a simple storage of ingested fat. This is seen in *Galleria*, in which the pupal fat consists of glycerides of higher fatty acids, quite unlike the waxes of the food (p. 330) [126, 127] and in the mealworm [10] and *Dermestes*.[166] The influence of the food is well seen in the larva of the beetle *Pachymerus*, which contains in its fat some 24 per cent. of lauric acid—far more than in most insect fats, though only about half that in the fat of the oil-palm kernels upon which it feeds [33]; also in *Blatella*, which has an iodine value of 51 when fed on coconut oil (7), 59 when fed on butter fat (27) and 64 when fed with lard (49) [131]; and in *Lucilia* larvae in which the fat has an iodine value of 140 when fed on fish fat with a value of 113, and 60 when given a fat with a value of 30.[203]

These fats, which are stored chiefly in the fat body (p. 239) provide most of the energy for growth and metamorphosis (p. 327) and during periods of starvation (p. 329). But nothing is known of the stages of their breakdown.

Carbohydrate metabolism—Sugar in the blood and tissues is probably the most readily available source of energy. Its importance is most obvious in the bee, in which the average level in the blood of foraging bees is 2·6 per cent., while values up to 4·4 per cent. (even 11·5 per cent. in one instance) may occur! It is a dextro-rotatory sugar, fermentable by yeast, and is presumably glucose. If its concentration in the blood falls below 1 per cent., the bee is unable to fly, but runs along with wings vibrating. Below 0·5 per cent. the bee becomes almost motionless.[13] During flight, the bee, weighing about 100 mg., will utilize sugar at the rate of about 10 mg. per hour.[94] Its normal store is exhausted in about 15 minutes, during which time it may fly some 5½ km. Its sugar disappears completely after a few hours of starvation, so that it must have constant access to the nectar of flowers or honey in the hive.[13] In most insects, as we have seen (p. 229), there is very little fermentable sugar in the blood. In the silkworm larva, for example, it averages only 0·022 per cent. This small amount disappears completely after an injection of insulin.[197]

In the larva of the bee, some of the carbohydrate ingested with the food is stored in the blood, partly as glucose and partly as a polysaccharide of some kind.[152] But carbohydrate is stored mainly in the fat body, in the form of glycogen. The importance of glycogen as a reserve substance varies in different groups. In the mature larva of the bee it may comprise 33·5 per cent. of the dry weight [171]; in the larva of *Gastrophilus* at one stage of growth it may reach 31 per cent. of the dry weight.[103] But in *Ophyra*,[173] *Calliphora*,[196] *Lucilia*,[54] *Bombyx mori*,[138] *Malacosoma*,[153] it is present only in small amounts—less than 5 per cent. of the dry weight. In the adult bee, glycogen forms from 0·3–0·9 per

cent. of the live weight.[13] In many cases this glycogen is doubtless derived from carbohydrate in the food. To what extent it may come from fat or protein is uncertain; but it has been noted that the silkworm may more than double its glycogen reserve in four days at the end of larval life, apparently by transformation of its fat or protein.[5]

The production of chitin (p. 19) is another aspect of carbohydrate metabolism. It is believed to be formed partly from glycogen and partly from protein.[196]

Protein metabolism—Proteins provide the chief structural elements of the muscles, glands and other tissues. They comprise some 2·2 per cent. of the fresh substance of the adult bee when newly hatched, 3·2 per cent. in the foraging bee with fully developed flight muscles.[101] Proteins may be transferred from one part of the body to another. In young bees the pharyngeal glands become greatly developed for the feeding of the brood; when these glands atrophy, the protein is transferred to the wax glands and the flight muscles [12, 82]; whereas in the queen termite and in queen ants, the disused flight muscles are utilized for the maturation of the gonads. In addition, a certain amount of protein is stored in the fat body (p. 239) and much is deaminated or converted into carbohydrate or fat and used for energy production. The fate of the waste nitrogen liberated in this process has been considered in Chapter XII.

If particular amino-acids are utilized for the production of some secretion, the composition of the proteins in the body as a whole may be greatly modified. This is well seen in the silkworm. In the mature larva the proteins consist of: glycine 10 per cent., alanine 8·7 per cent., tyrosine 4·3 per cent., valine 1·7 per cent., leucine 4·8 per cent., aspartic acid 1·6 per cent., glutamic acid 3·5 per cent., phenylalanine 2·4 per cent., proline 1·5 per cent. The silk of the cocoon is composed chiefly of tyrosine, glycine and alanine (p. 330), with the result that the protein of the silkworm moth is greatly altered and consists of: glycine 3·5 per cent., alanine 3·2 per cent., tyrosine 1·6 per cent., valine 1·7 per cent., leucine 8·5 per cent., aspartic acid 2·7 per cent., glutamic acid 5·7 per cent., phenylalanine 2·7 per cent., proline 4·0 per cent.[1]

Chemical changes during growth—Little is known of the changes which take place in the separate tissues during development; what information exists is derived from analyses of the body as a whole. By this means only the grossest alterations are detected; if the breakdown of a given substance in one part of the body is compensated by its synthesis in another, analysis of the whole insect may show no change at all.

In the egg the chief reserve substance which provides the energy for growth, the 'work of development',[173] is usually fat. In the silkworm egg, fat yields about two-thirds of the total energy,[55] falling from 8·1 per cent. to 4·4 per cent. of the wet weight.[183] The remaining third is provided by glycogen, which falls from 2 per cent. to 0·74 per cent. of the wet weight,[183] and probably protein.[55] In *Melanoplus* eggs, higher fatty acids compose 17–22 per cent. of the dry weight at the time of laying; these diminish rapidly during the periods of growth before and after hibernation, and at the time of hatching less than half remains.[168]

During larval growth there is usually no striking change in composition until shortly before pupation. In *B. mori* the newly hatched larva contains

about 4 per cent. of fat; this quickly falls, and remains at a low level; only in the last stage is fat stored extensively.[102] In *Malacosoma* (Lep.), fats compose only 0·66 per cent. of the dry weight at hatching; at the end of larval life they increase rapidly to 28·8 per cent.[153] In *Lucilia*, fat increases in the same manner up to 30 per cent.[203] This great increase in fat may result in a relative fall in other constituents, such as water [203] and protein; in *Malacosoma* larvae the total nitrogen falls from 15·1 per cent. of the dry matter at hatching, to 10·5 per cent. in the full grown caterpillar.[153] In those insects in which it is stored extensively, glycogen seems to be accumulated more gradually during larval life, as in *Apis* [171] and *Gastrophilus*.[46] In *B. mori* there is a sexual difference, accentuated in the adult but already noticeable in the larva; the females storing more glycogen, the males more fat.[190]

TABLE 3

Day of larval life		*% dry matter*	*% glycogen*	*% fat*	*% nitrogen*
1		22·9	—	—	—
2		—	2·5	1·5	2·9
3		17·8	—	—	—
4		—	5·6	—	—
5		20·0	—	3·6	1·5
6		—	6·6	—	—
Day of pupal life	*Weight in mg.*				
1	147–176	23·0	6·2	4·1	1·2
3–4	142	22·0	5·2	3·7	1·6
7	123	19·2	3·0	2·8	1·7
12	113	15·2	0·5	1·5	1·7
13 (hatched bee)	111	14·8	0·5	0·9	2·0

Table 3 summarizes the changes in composition which take place in the larva of the worker bee.[171] During the first four days this larva receives a diet consisting of 58 per cent. protein, 8 per cent. fat and 10 per cent. sugar; after the fourth day the diet consists of 28 per cent. protein, 4 per cent. fat, 45 per cent. sugar.[105] Storage of glycogen takes place in the second period, when carbohydrate predominates in the food.[139] By the end of larval life, about 50 per cent. of the dry weight consists of reserve substances.

The same Table shows the changes in composition in the pupa. This has a wet weight of 150 mg., and the stage lasts 13 days; during this time it consumes 8·4 mg. of glycogen (about 95 per cent. of its store) and 4·9 mg. of fat (about 75 per cent. of its store); the glycogen, as in other insects, being used up first.[171] Most insects consume chiefly fat in the pupal stage; in *Ophyra cadaverina*, over 90 per cent. of the substance consumed is fat [173]; in *Calliphora*, the fat content falls from 7 per cent. of the dry weight at the beginning to 3 per cent. at the end.[196] We shall see later that during pupal development the energy production follows a U-shaped curve; it is high at the beginning and end of development, with a low period between (p. 345). The rate of disappearance of fat follows the same course in *B. mori*,[138] *Ophyra*,[173] *Tenebrio*,[54] &c. In *Lucilia* there seems to be a selective utilization of the unsaturated fatty acids, saturated acids remaining constant.[54] In the pupa of *Galleria*, reducing

substances follow the same type of curve.[40] Free amino-acids fall steadily as histogenesis proceeds (in *Deilephila* [83] and other Lepidoptera [37] and in *Lucilia* [54]). In the silkworm pupa altogether about 8·6 per cent. of the dry substance, or 12·1 per cent. of the total energy is used up.[55]

The extent to which protein contributes to the energy metabolism of the pupa varies. *Bombyx mori* and the Sphingid *Deilephila* afford a striking contrast in this respect. In *B. mori* about half the protein of the mature larva is used to make the cocoon, half the total dry weight being lost at pupation; whereas *Deilephila* loses only one quarter of the dry weight. During pupal development *Deilephila* covers 20 per cent. of its energy metabolism from fat, the remaining fraction being chiefly protein; whereas *B. mori* uses fat for nearly 50 per cent. of its total energy, the remainder being covered chiefly by carbohydrate.[83, 138] In all groups there is a great reduction in the water content at pupation. In *B. mori* 100 larvae contain 37·4 gm. of solids, 139·5 gm. of water; 100 cocoons and pupae contain 33·9 gm. of solids and 83 gm. of water.[138]

Chemical changes in starvation—During starvation the relatively small amounts of carbohydrate are first consumed. In the mealworm larva starved at 30° C., the glycogen falls from 2·04 per cent. of the wet weight to 0·68 per cent. in a week [133]; in Odonata it falls from 0·2 per cent. to zero.[169] Proteins may be used extensively—as in *Dytiscus*,[142] adult *Deilephila* which use up 41 per cent. of their protein before death,[84] *Melolontha* which uses 22 per cent. and *Geotrupes* which consumes 20 per cent.[169] Or the protein metabolism may be very limited—as in *Periplaneta*,[142] in Odonata, where the protein content falls from 53·4 per cent. to 51·7 per cent. of the dry weight, or *Bombus*, where it falls from 58·3 per cent. to 52·1 per cent.,[169] or in *Apis*, in which, at least at 23° C., there appears to be no utilization of protein at all during fasting.[101]

But fat is always the chief reserve substance that is drawn upon. In the mealworm larva, fat comprises about 14·8 per cent. of the wet weight; and when starved at 30° C. about half of this is used up in one month.[133] In adult *Deilephila* 70 per cent. of the fat is broken down by the time death occurs [84]; *Melolontha* adults use up 85·6 per cent. of their fat before death, *Geotrupes* about 75 per cent., and in Odonata the fat content falls from 11·7 per cent. of the dry weight to 5·9 per cent.[169]

Similarly, during hibernation, fat forms the chief reserve. In *Culex* adults, the mean wet weight fell from 3 mg. in September to 2 mg. in April; the fat fell from 0·91 mg. to 0·13 mg.; solids other than fat remained approximately constant. Meanwhile, chiefly as the result of this loss of fat, the proportion of water rose from 54 per cent. to 65 per cent.[29] In some insects, on the other hand, although much fat is laid in before the winter, very little is consumed until the spring. Full-grown larvae of *Pyrausta nubilalis* contain fat equal to 26·5 per cent. of the wet weight. But during the seven months diapause there is almost no fall. Breakdown occurs shortly before pupation, the pupa containing 9·7 per cent. In this process there is no preferential utilization of unsaturated fats, the iodine value ranges from 76 to 81 throughout.[184] And adults of *Leptinotarsa* increase their fat from 2·6 per cent. of the wet weight in September to 12·6 per cent. in November; by March this has fallen only to 11·6 per cent.; the rapid fall to 3·5 per cent. takes place only with the renewal of activity.[27]

SOME CHEMICAL PRODUCTS OF INSECTS

Systematic accounts of the many kinds of glands which open upon the surface of the insect body are given by Imms (1935) and by Weber (1933). Here we shall merely attempt to give some idea of the extraordinary range of the chemical products of these glands, products which in several cases are materials of commerce.

Foremost among these is **silk,** which is secreted not only by the labial spinning glands of Lepidoptera (Fig. 261), Hymenoptera, &c., but by the accessory tubes of the genital organs in *Hydrophilus*, by the tarsal glands of Embioptera (Fig. 262) and Empidae, and by the Malpighian tubes of certain Coleoptera and Neuroptera (p. 315).[117] In Lepidoptera a silk fibre is formed by each labial gland. As these fibres pass down the common duct they go through a muscular press which squeezes them together and transforms the two cylindrical strands into a flattened ribbon. Accessory glands upon the common duct perhaps secrete a cementing substance which holds the two fibres together.

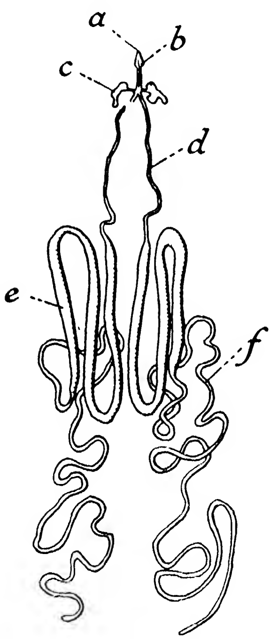


FIG. 261.—Spinning glands of silkworm (*after* LESPERON)

a, spinneret; *b*, press; *c*, Lyonnet's glands; *d*, silk ducts; *e*, reservoir; *f*, secreting gland.

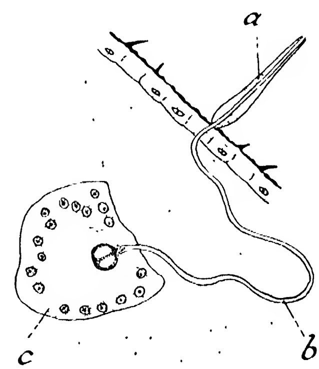


FIG. 262.—Silk gland from tarsus of *Embia* (*after* LESPERON)

a, spinning hair; *b*, duct; *c*, gland with ampulla containing silk substance.

The mechanism of solidification of silk is not understood; it is not due simply to drying, for it will take place under water.[62] Chemically, silk consists of 70–75 per cent. of a tough elastic protein, 'fibroin' (probably secreted by the posterior division of the gland) in which the chief amino-acids are glycine 36 per cent., alanine 21 per cent. and tyrosine 10 per cent.; and 20–25 per cent. of a gelatinous protein 'sericin' (probably secreted by the middle division [188]) which forms a covering layer. Sericin is readily soluble in warm or soapy water; it is composed mainly of serine 5·8 per cent., alanine 9·2 per cent., and leucine 5·0 per cent.[62]

Wax is secreted in the form of powders, threads or thin plates by the dermal glands of many bees,[50] Hemiptera [192] and other insects. All the insect waxes (lac wax, coccerin, psylla wax, beeswax, &c.) are mixtures in varying proportions

of (i) even numbered alcohols ranging from C_{24} to C_{36}, (ii) even numbered *n*-fatty acids from C_{24} to C_{34} and (iii) paraffins, always with an odd number of carbon atoms, from C_{23} to C_{37}. The various waxes differ only in the proportions of these products, and in the extent to which the alcohols are free, or combined as true wax-esters with the fatty acids.[31]

Waxy or oily secretions are associated with the spiracles of aquatic insects (p. 200). Glands secreting a fatty covering which opposes evaporation occur in *Epiphragma ocellaris* and other Tipulid larvae from dry wood,[99] as well as in the cockroach.[52, 150] Dermal glands along the articulations of Odonata [162] and other insects [193] perhaps produce an oil which lubricates the joints. The mandibular glands of caterpillars produce an oily secretion; in the larva of *Cossus* they are particularly active, and produce up to 0·4 gm. of a strongly smelling yellow oil, which contains a mixture of hydrocarbons, some with sulphur in organic combination.[87]

Lac is a complex of resinous substances, wax, colouring matter, &c., secreted by various Coccids, particularly *Tachardia*, *Gascardia* and *Lecanium*. Its composition varies considerably with the food plant.[119]

Venoms are secreted by the modified accessory glands of female Aculeata. The venom of the bee is produced solely by the so-called 'acid gland' of the sting (Fig. 263).[89] It seems to contain several toxic constituents which can be isolated by different methods, some of them being nitrogen free.[59] Histamine present may account for the cutaneous reaction to the sting [130]; but the chief toxic fraction, 'apitoxin', seems to be a dialysable protein of low molecular weight with an isoelectric point at about *p*H 8·7.[81, 178] Associated with the sting is a second gland, the 'alkaline' or Dufour's gland, which has nothing to do with the toxic action. It perhaps secretes an oily liquid which serves to clean and lubricate the sting [89, 163] or to line the top of the cell,[163] or to secure the egg to the cell wall.[156, 187]

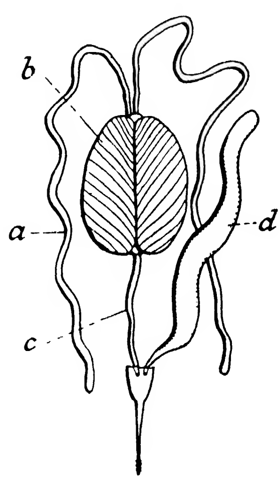


Fig. 263.—Poison glands of the wasp *Vespa germanica* (*after* Bordas)

a, acid gland; *b*, poison sac; *c*, duct of acid gland; *d*, alkaline or Dufour's gland.

In solitary Hymenoptera (Pompiliids, Sphegids, &c.) the venom is used for paralysing the prey. Spiders or caterpillars when stung may remain motionless for months, although the heart continues to beat. In Cynipids the venom provokes the formation of plant galls.

Myrmicine and Dolichoderine ants produce venom of an unknown nature, perhaps a toxic protein [172]; but among Camponotine ants the chief constituent of the venom is formic acid. In *Formica rufa* from different localities formic acid may range in concentration in the venom from 21 per cent. to 71 per cent. amounting in some cases to 18 per cent. of the total body weight.[172]

Other well-known poisons produced by insects are: *cantharidin* ($C_{10}H_{12}O_4$), which may form 3–10 per cent. of different Meloid beetles, occurring largely in the blood, but also in the accessory glands of the genital tract [59]; the intensely toxic *arrow poison* obtained by bushmen in the Kalahari Desert from the larva of the beetle *Diamphidia locusta*, which appears to be a toxic saponin [62]; and

the *urticating substances* secreted into the hollow cavities of the fragile detachable barbed hairs of many caterpillars [70]; these are of unknown nature, but they are often thought to be related to cantharidin.

Stink glands, perhaps with a repellant function, occur in Hemiptera and other insects,[104, 192]; and **scent glands** play an important part in reproduction among Lepidoptera (p. 384), and in the social behaviour and orientation of ants and bees.[92] But nothing is known of the chemistry of these products. The larva of the Chrysomelid *Melasoma populi* has a series of segmental reservoirs into which it secretes salicylaldehyde, an end-product no doubt of the glucoside salicin in the food (Fig. 264) [66]; and the adult of the beetle *Aromia moschata*, feeding on the same group of food plants, eliminates salicylic acid in the form of an ester, through a pair of glands on the hind coxae.[90, 170] Eversible pouches, essentially similar to those of *Melasoma*, form the 'osmeteria' of *Papilio* larvae. These also secrete strongly scented substances; perhaps they arose for the same purpose of eliminating poisonous compounds from the plants (Aristolochiaceae, Umbellifera, &c.) on which these caterpillars feed.[160, 194]

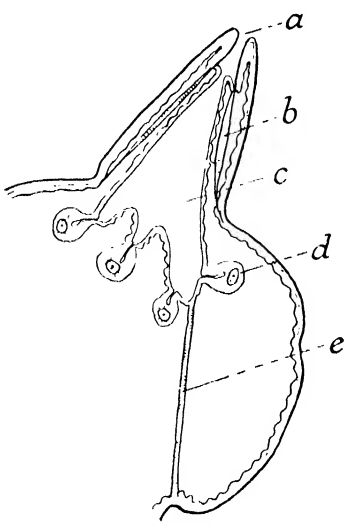


FIG. 264.—Section of segmental gland in *Melasoma populi* (*after* GARB)

a, pore; *b*, muscle closing the pore; *c*, reservoir for secretion; *d*, unicellular gland with intracellular duct; *e*, retractor muscle.

There are **other substances** occasionally secreted by insects. The caterpillar of *Notodonta concinnula* emits strong hydrochloric acid.[43] The full-grown caterpillar of *Dicranura vinula* will eject 0·5 gm. of 40 per cent. formic acid [145]; this it incorporates with the secretion of the spinning gland to produce the hard and waterproof material of the cocoon.[116] This same species, when it hatches as an imago, softens the cocoon with an alkaline solution, said to contain 1·4 per cent. of potassium hydroxide.[115] The pygidial glands of *Carabus* secrete butyric acid.[129] Some Mycetophilid larvae secrete onto their web droplets of 0·15 per cent. oxalic acid, which serves to kill the insects, &c., on which they prey, when these come in contact with it.[125] The corrosive vapour discharged by the bombardier beetle *Brachinus*, boils between 8° and 15° C. and seems to contain oxides of nitrogen.[62] And the vapour discharged by Paussid beetles contains free iodine.[62]

PIGMENT METABOLISM

Attention has always been attracted to the pigmented constituents of insects. In some cases these are, perhaps, substances of physiological importance; but the majority seem to be merely by-products of metabolism. Yet since they furnish the colour patterns they are not always without biological significance.

Melanin pigments are generally incorporated in the substance of the cuticle (p. 29); though in some cases, such as *Dixippus* (p. 337), the dark granules of pigment within the epidermal cells are said to belong to this group.

They range in colour from yellow to black, and are characterized by their complete insolubility in ordinary solvents. Chemically they arise from the polymerization of certain indol ring compounds, which are themselves ultimately derived, by oxidation and ring closure, from the amino-acid tyrosine.[3, 182] Tyrosine (monoxyphenylalanine) is oxidized in the presence of the enzyme tyrosinase; the first product of the reaction being 'dopa' or 3–4 dioxyphenylalanine. The subsequent stages in oxidation are much more readily accomplished; a very weak tyrosinase will act as a 'dopa-oxidase'; and various other aromatic substances bearing one or more phenolic groups can serve as raw material or 'chromogen' for melanin formation.[36]

The immediate precursor of melanin in insects seems usually to be a dioxybenzene derivative of this type; either 'dopa' itself, as in the elytra of *Melolontha*,[157] dioxyphenylacetic acid, as in the elytra of *Tenebrio*,[157] or some related compound. These substances occur also in the cocoons of Lasiocampids and Saturniids, being derived from the secretion of the spinning glands and Malpighian tubes. If the cocoons are kept dry they remain pale; but if moistened, the small amount of oxidase present can react with the chromogen, and the cocoons darken.[45, 148]

Thus the deposition of melanins requires the interaction of tyrosinase, chromogen and oxygen. The necessary oxygen is obtained through the tracheal system, not from the air in contact with the surface.[61, 86] The process is arrested in nitrogen, as in *Leptinotarsa*,[71] *Pyrrhocoris*,[86] the puparium of *Calliphora*[61]; and it is arrested if the tyrosinase is destroyed, as when the elytron of *Leptinotarsa* before pigmentation is heated at 70° C. for 1 minute.[71] Blackening is prevented also if the blood stream is interrupted, as in *Pyrrhocoris*,[86] and the Coccinellid *Epilachna*.[177] In *Tenebrio* the chromogen is almost absent from the cuticle of the newly emerged beetle; it becomes abundant later, reaching a concentration of 5 per cent.[157] In general it seems to be the localization of chromogen which determines the distribution of the pigment patterns; chromogens are absent from white areas of cuticle,[157] whereas tyrosinase is everywhere. The pattern of *Leptinotarsa* is little affected if the unpigmented elytron is immersed in tyrosinase; it is entirely blackened if placed in tyrosine[71]; the same applies to the developing wings of *Pieris*.[140] During the development of the wings in Lepidoptera, the chromogen appears to be deposited in the substance of the scale.[151] But the process as a whole is controlled apparently by the nervous system,[61] perhaps through the medium of hormones; and the changes even in the surface layers of the cuticle are under the control of the cells below (p. 29).[200] In *Tenebrio*, darkening occurs only in the living insect.[157]

The production of melanin is sometimes regarded as a mechanism for disposing of toxic phenols arising as breakdown products in metabolism.[36] In some cases its distribution seems to be related with the intensity of metabolism in the subjacent tissues: it occurs in the cuticle over the muscle insertions in many insects,[186] and over the fat body and pericardial cells of Vespids (Fig. 265)[8]; and the blood of fly larvae blackens most readily immediately before pupation.[44] During the past century, melanic races have arisen among many species of Lepidoptera, particularly in industrial regions.[80] This has sometimes been regarded as a direct effect of city fumes upon metabolism[80]; sometimes as a mutation induced by contamination of the larval food plants

with such metals as lead and manganese.[78, 179] In any case it is the pigment precursors which are increased in the tissues of these insects; there is no difference in the oxidase content.[80]

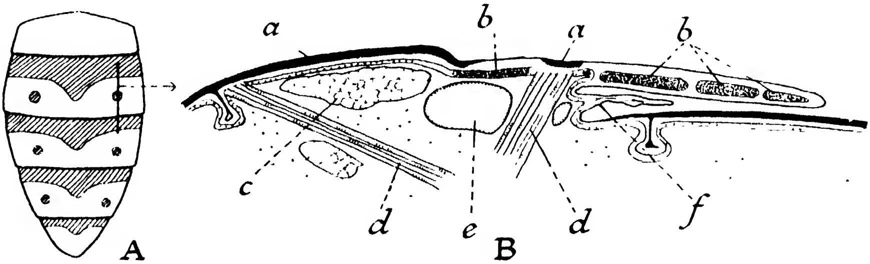


FIG. 265

A, pigment pattern on abdomen of *Vespa*. *B*, longitudinal section of tergite and intersegmental region to show the distribution of melanin and pterine pigments in relation to the underlying structures (*after* BECKER). *a*, melanin in cuticle; *b*, pterine in thickened epidermis; *c*, fat body; *d*, muscles; *e*, tracheal air sac; *f*, intersegmental membrane.

Pterine pigments are widely distributed in nature, being recognized in hay, and in the liver and urine of mammals. Possibly they are mere end products of metabolism; perhaps they have some unknown function (one of them forms an essential factor or vitamin in the diet of rats). In colour they may be white (leucopterine), yellow (xanthopterine and others) or red (erythropterine). The natural pigments are always mixtures of several pterines.[158] They are particularly characteristic of the Pieridae,[91a] the wing pigments of which, unlike those of most Lepidoptera, do not fluoresce when exposed to ultra-violet light.[32] But they occur also in Neuroptera (*Ascalaphus*), Syrphidae, some Cicadidae, many Hymenoptera such as *Vespa*, and doubtless in other insects.[7, 158]

The pterines are related chemically to the purines, such as uric acid. Leucopterin, which occurs in several isomeric forms, has the empirical formula $C_{19}H_{19}O_{11}N_{15}$, being made up of three imino-pyrimidine rings of guanine type; but its structure has not been definitely determined. It is perhaps an oxidation product of xanthopterine ($C_{19}H_{19}O_7N_{15}$). In spite of their structural similarity, however, it is doubtful if these substances are related to uric acid in metabolism; they never occur in the urine, and uric acid is never present in the wings.[198] The pterines are deposited in the substance of the epidermal cells, or in the lumen of scales or hairs; they are usually laid down where the metabolism in the adjacent region is low. In *Vespa*, for example, they occur within the rigid fold of cuticle which overlies the intersegmental membrane, over the large tracheal air sacs, and in the distal segments of the limbs (Fig. 265).[8] In the nest-mother of wasps in the latter part of the year concentric rings of dark pigment may be deposited in the yellow regions of the abdominal folds. These appear to be Liesegang rings of precipitation; perhaps the sequel to some injury.[9]

Carotinoid pigments or lipochromes are almost universal in plants, and they are commonly absorbed by insects and accumulated in the blood or tissues.[42] Carotin itself is most readily absorbed; in the blood of *Leptinotarsa* it occurs at a concentration of 14 mg. per cent., equal to that in many leaves[107]; and it provides the red and yellow colouring of the bug *Perillus*

which preys upon this insect.[141] It is deposited in the cellular tissue of the elytra of *Melasoma* and *Harmonia* [111] and in the fat body of *Pyrrhocoris*.[161] (The red and orange pigments in the epidermis of *Pyrrhocoris*,[86] *Rhodnius* [200] and other bugs are not carotinoids; their nature is unknown). Carotin is absorbed into the blood of many caterpillars, such as Noctuids [134] and Pierids.[67, 127] It is present in the wings of *Melanargia* (Lep.).[180] In *Dixippus* it may be transmitted by the female to the eggs and so to the offspring.[185]

Xanthophyll, the dioxide of carotin, on the other hand, is much less readily absorbed. It is absent from *Leptinotarsa* [141] and from Noctuid larvae (*Agrotis*, *Caradrina* [134]); but it is present in the blood and epidermis of Pieris larvae.[127]

The coloration of the silk cocoons of *Bombyx mori* is also due to carotinoid pigments from the food [191]; and the different silkworm races with distinctive colouring of the cocoon differ in the proportions of xanthophyll and carotin which are transferred from the gut to the spinning gland, and in the rate and time at which this transfer is achieved.[95, 127, 128]

Whether carotinoids play a part in insect physiology is not known. In mammals, vitamin A is a derivative of carotin, and a related derivative forms the visual purple of the retina.

Anthocyanins and flavones, which are important flower pigments, are not very common among insects. The beetle larva *Cionus* contains in its fat body granules of anthocyanin from the *Verbascum* on which it feeds [91]; and the vermilion colour of the Aphid *Tritogenaphis* is perhaps an anthocyanin.[141] The whitish wing pigment of the Satyrine butterfly *Melanargia* is a flavone identical with that present in the grass *Dactylis glomerata* on which the larva feeds [180]; and flavones contribute to the cocoon colours of some silkworm races and are present in the blood and epidermis of *Pieris* larvae.[127]

The **green pigments** of insects have often been thought to be derived from altered chlorophyll (see p. 232).[146] But chlorophyll cannot be detected in the green blood or epidermal pigment of Noctuid larvae,[134] *Pieris* [127] or *Locusta* [56]; while *Dixippus*, *Mantis*,[185] *Locusta*,[56] and *Caradrina* [134] develop their normal green pigment when no chlorophyll is present in the food. The green pigment between the wing membranes of *Pieris* is a 'chromoproteid', the pigmented component of which is very closely related with the mammalian bile pigment biliverdin.[199]

There are many **other pigments** in insects the chemical nature of which is quite unknown. The red and orange pigments of Vanessid butterflies have been thought to be derived from chlorophyll, but this conclusion is not based on chemical evidence.[118] The eye pigments of *Ephestia* and other Lepidoptera, though often dark brown or black, are not related with the melanins [30, 143]; and the chemistry of the eye colours of *Drosophila* is equally unknown.

Colour change—Some insects can change in colour in response to environmental stimuli. In all such cases, with the exception of the Mantids and the Phasmid *Dixippus* to be described below, the change results from alterations in the quantity of pigment formed and consequently it is not immediately reversible.

Temperature is one such stimulus. We have seen that temperature acting in the early pupal stage of butterflies may determine the seasonal form (p. 60). The effect can be produced by applying the warm or cold stimulus to the head alone. Perhaps the head contains a 'pigmentation centre', which regulates

either the rate at which the melanin-forming process proceeds, or the quantity of chromogens capable of yielding this pigment.[69] The formation of melanin in the wasp *Habrobracon* decreases if it is reared at a high temperature [155]; the females of this insect are darkened if the pupa is exposed to extremes of heat or cold; and this effect is transmitted, apparently through the plasma, to the next generation.[96] And in the bug *Perillus* both melanin and carotin are at a maximum at low temperature (18–24° C.), at a minimum at high (29–35° C.).[107]

Humidity has been regarded as the factor determining the wet- and dry-season forms of the butterfly *Hestina assimilis*; for the application of water to one pupal wing will produce insects with wings of the heavily pigmented wet-season form on that side, and the pale dry-season form on the other [132]; but it is possible that this again may be an effect of temperature. In the highly variable *Locusta migratoria*, bright green hoppers develop only when fed with succulent moist food in a very humid atmosphere.[56]

Illumination is the chief factor controlling colour change. This is well seen in Vanessid and Pierid pupae, which are dark or pale according to their surroundings.[145] The pupal colour of *Pieris* is given by patches of melanin in the superficial layer of the cuticle, by a white pigment in the epidermal cells, and by the green colour of the deeper tissues.[53] The colour varies with the quantity of the black or white pigments, the formation of which is influenced by the illumination of the larva as it rests before pupation. Exposure to green, yellow and, particularly, orange light at this time causes a suppression of the white and black pigments so that the pupae appear plain green (Fig. 266). This influence is transmitted to the offspring, which tend to produce green pupae even under ordinary illumination,[53] an effect which is exaggerated after two generations have been reared in orange light.[79] Ultra-violet light increases the formation of black pigment.[25] Substantially the same effects are obtained with *Vanessa*, but here black pigment is suppressed, and white enhanced, by infra-red rays, which result in whitish pupae.[25] These effects are still produced if the larval eyes are covered with black pigment [145]; but either the eyes or some centre in the head control the response, for it is eliminated if the larva is decapitated or if the eyes are cauterized.[25]

A

B

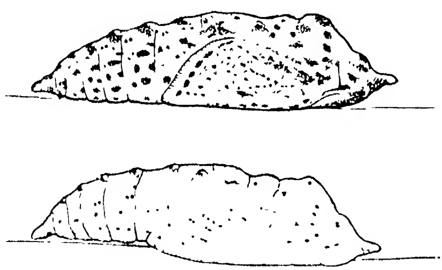


FIG. 266.—Effect of background on coloration of pupae of *Pieris brassicae* (*from photograph by* DÜRKEN)

A, whitish grey pupa with much black pigmentation: black and red background. *B*, pure green pupa with black pigment greatly reduced: green or orange background.

Hoppers of *Locusta migratoria* can also adapt their colours from dirty white to yellow, brown or black according to the background [56]; the changes being dependent on the quantities of orange-yellow and black pigments formed. The amount of black pigment seems to depend on differences in the intensity of the light incident from above and reflected from below, acting upon the eyes.[88] The production of orange-yellow pigment is stimulated by rays with wave-lengths within the limits of yellow (5,500–6,000 Å); it is inhibited by

blue and violet light (5,000–4,500 Å).[88] This same species shows also a striking colour change, with conspicuous black and orange markings, when it enters the gregarious migratory phase of its existence. This change is associated with intense muscular activity: hoppers of the gregarious type kept singly and undisturbed revert to the solitary phase; whereas if they are continually agitated they retain, at least in part, their 'gregaria' type of colouring. Perhaps under these conditions some diffusible substance is produced which controls the pigmentation. If so, it seems to be passed on by the female to her offspring, for the 1st instar hoppers of 'gregaria' show their characteristic coloration within an hour after hatching.[56]

The Phasmid *Dixippus* has a series of colour forms from green to brown, depending upon the quantity of brown, orange, yellow and green pigment granules in the epidermal cells—a 'morphological' colour change. And each form, except the green variety, has its own range of colours, brought about by the dispersion or clumping of the brown and orange pigments within the cells—a 'physiological' colour change (Fig. 267).[68, 154] Normally the insect is dark at night and pale by day, and this change will continue for several weeks in complete darkness. This diurnal rhythm is absent in insects kept in the dark from the time of hatching; it is induced by periodic illumination. Insects illuminated at night will become pale; and a reversed rhythm, persisting in continuous darkness, can be induced by reversed illumination.[154] High humidity also causes darkening; dry air causes pallor.[68] In Mantids, also, there is a colour change in the epidermal cells which takes place at night, simultaneously with the assumption of the superposition arrangement in the lateral regions of the compound eyes (p. 114).[64]

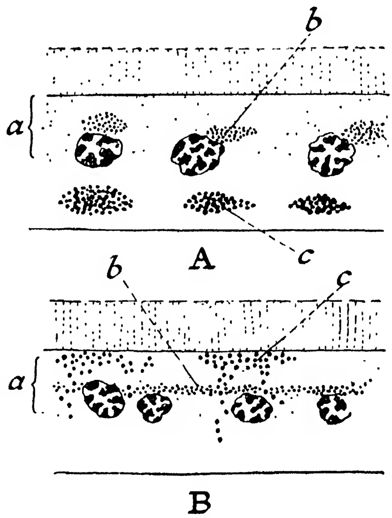


FIG. 267.—Pigment movements in epidermal cells of *Dixippus*

A, light position; B, dark position. a, green and yellow pigments; b, orange red pigment; c, brown pigment. (*After* GIERSBERG.)

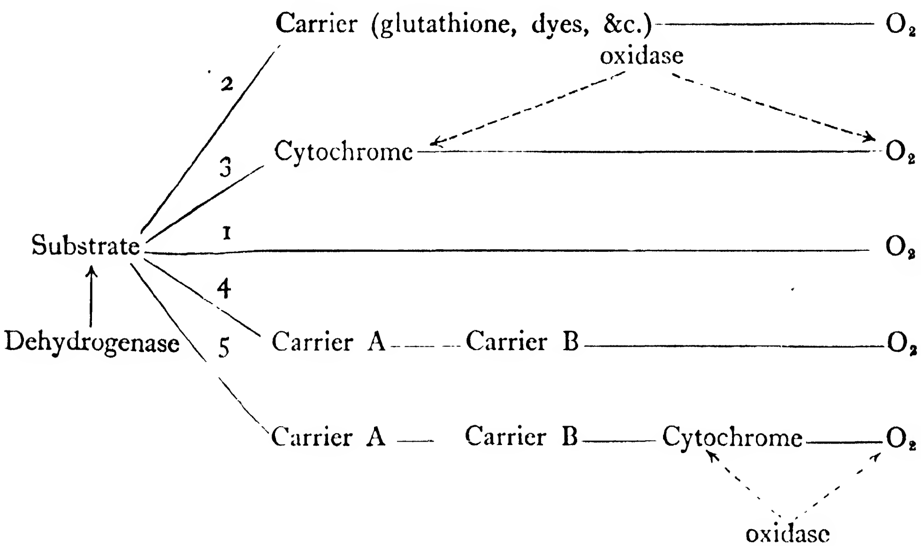
These effects are indirect; they apparently depend on some nervous stimulus reaching a centre in the head and leading to the production, perhaps in the corpora allata or other glandular organs, of a hormone which causes the pigment movements in the cells.[2, 68] The epidermal cells are not innervated; and if pieces of cuticle and epidermis are transplanted from one insect to another they change colour simultaneously with their new host.[93] It is worth noting also that extracts from the head of *Dixippus* and other Orthoptera will cause contraction of the chromatophores of Crustacea.[75]

The effect of light on *Dixippus* is exerted solely through the eyes; section of the eye stem eliminates the response.[2] It is determined by the contrast between the background and the surroundings; if the ventral half of the eye is blackened, darkening takes place; whereas all colour change ceases if the eyes are completely covered. Green is the most effective component of ordinary daylight in inducing the response; but if light of equal energy content is used,

ultra-violet is the most effective.[147] The 'morphological' colour change in *Dixippus* seems to be brought about by the same stimuli acting over a greater length of time.[2, 68]

RESPIRATORY METABOLISM

Tissue respiration—The organism derives its energy from the oxidation or combustion of organic substances. But at the temperature of the body these will not unite directly with molecular oxygen; intermediaries or respiratory catalysts of one sort or another are necessary. Some of the catalysts which have been recognized in other organisms are indicated in the following scheme. In this scheme oxidase is sometimes called the 'Atmungsferment' or 'respiratory enzyme'; carrier A is called 'coenzyme' and carrier B is 'flavo protein' or 'yellow enzyme'.



In every case it is necessary for the organic substrate to be acted upon by a specific enzyme or dehydrogenase, which 'activates' a part of its hydrogen so that it can readily be oxidized. In a few cases (scheme 1) it may then be oxidized directly by molecular oxygen. Usually, oxygen and hydrogen can be brought together only through a system of carriers. Glutathione, which can be reduced by active hydrogen and oxidized by molecular oxygen (scheme 2), is one such carrier; cytochrome is another; but cytochrome requires a respiratory enzyme or oxidase to catalyse its oxidation (scheme 3). In some cases a chain of carriers must be interposed between substrate and oxygen, as shown in schemes 4 and 5.[47]

Cytochrome, consisting of a mixture of three haemochromogen compounds, plays perhaps the most important part in the respiratory mechanism of the cells in insects. It is present in the muscles in proportion to their activity; there is little in the thorax of wingless insects, much in the bee, and much in the leg muscles of active running forms. It can be seen in living muscles to undergo continual oxidation and reduction: in the resting thoracic muscles of *Galleria* it is oxidized, during activity it is partly reduced.[100] Glutathione

is present in various insect tissues [58]; but probably it is responsible for only a small part of the oxygen transfer. Under experimental conditions, reversibly oxidizable dyestuffs, such as methylene blue, will increase respiration by acting as subsidiary carriers of oxygen to the reducing systems. It is possible that some of the pigments associated with the tracheal cells and tracheal epithelium, which have been shown to be reversibly oxidizable, function in this way (p. 189).

That there are several oxidizing systems at work is shown by experiments with the eggs of *Melanoplus* (p. 9). If the actively developing egg is treated with potassium cyanide (which is believed to put out of action the cytochrome-oxidase system), the respiration is reduced to a minimum; whereas respiration during diapause is unaffected.[19] Methylene blue will stimulate the respiration of eggs in diapause, but not active eggs. And respiration of active eggs is depressed by carbon monoxide, while eggs in diapause are quite resistant.[22] That part of the respiration which is sensitive to cyanide is dependent on the intact structure of the embryo, whereas the cyanide insensitive fraction, like the respiration in diapause, in unaffected by breaking up the egg.[20] At all stages of pupal development in *Drosophila*, the oxygen consumption is strongly inhibited by carbon monoxide. This effect is to some extent prevented by exposure to light. Perhaps the light causes the dissociation of the chemical compound which carbon monoxide forms with the iron-containing respiratory enzyme.[202]

Catalase—The transfer of hydrogen to molecular oxygen reduces the latter to hydrogen peroxide, and it is probably the function of the enzyme catalase,

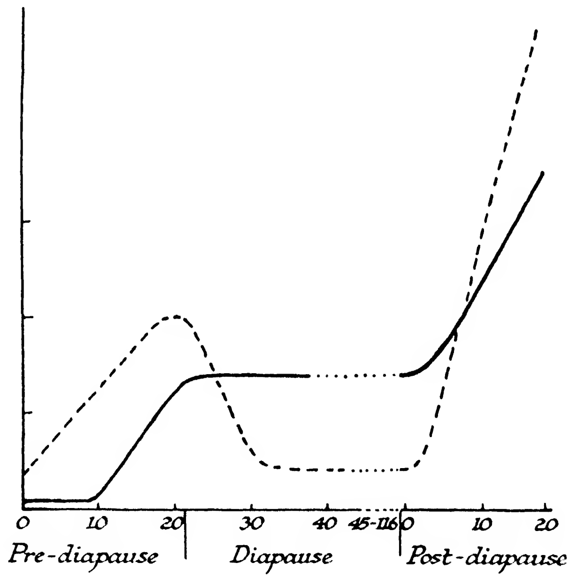


FIG. 268.—Catalase activity (continuous line) and respiratory rate (broken line) in eggs of *Melanoplus* from laying to hatching

Abscissa: days of development (*after* WILLIAMS).

which is always present in the tissues, to prevent the accumulation of hydrogen peroxide by decomposing it into oxygen and water. But there seems little

correlation between the catalase content of insects and the intensity of their respiration. Catalase increases during feeding and activity, and decreases during starvation (in grasshoppers [15] and *Leptinotarsa* [159]). But it is not markedly reduced in hibernating grasshoppers, when the respiration falls [15]; it does not follow the normal U-shaped curve of respiration in the pupa of *Leptinotarsa* [58a]; and in the eggs of *Melanoplus* the catalase content continues to rise, and remains at a high level, long after the insect has entered diapause, and the respiratory rate has fallen and mitotic activity has ceased (p. 10) (Fig. 268).[201]

Respiratory metabolism—The rate at which these processes of oxidation take place in the living body, varies within wide limits. Figures are available for many species of insects [113]; but the values recorded depend to so great an extent upon muscular activity, external temperature, tissue growth, and a host of other factors, that such figures do not afford a true measure of the intrinsic differences which must exist between different species. Even values obtained with the insect at rest, at the same temperature, are not strictly comparable; because the tissues of different species, as we shall see, are adapted to different ranges of temperature; some can maintain their metabolism and remain active at a lower temperature than others; and if tested at a low temperature will appear to have a higher metabolic rate. The important effects of temperature on respiration will be considered in the next chapter; here we shall examine the effects of other factors.

The kind of differences which exist are well seen among Ephemerid nymphs. Anaesthetized with urethane at 10° C., these had an oxygen uptake in c.mm. per gm. per hour as follows: *Ephemera vulgata* 278, *Coenis* sp. 290, *Ephemera danica* 370, *Ecdyonurus venosus* 604, *Ephemerella ignita* 950. These figures seem to bear some relation to the habitat of the species, the two first named species, which live in ponds, having a lower rate of metabolism than the last three species, which live in streams.[60] The reason for this difference is not clear; there is no evidence in this case that it depends upon adaptation to different temperatures.

Within the same species there may be a sexual difference. In *Drosophila* adults, under the same conditions, the males use about 2·8 mg. O_2 per gm. per hour, the females about 3·4 [114]; whereas in the pupae the metabolism of the males is greater.[144] Larvae and pupae of *Tenebrio* show a diurnal rhythm in metabolism; the oxygen uptake being greatest at about 2 a.m., least about 3 p.m.[135] As in mammals, a protein meal seems to have a 'specific dynamic action' increasing the rate of metabolism more than fats or carbohydrates. Thus cockroaches at 15° C. used oxygen at the rate of 101–113 c.mm. per gm. per hour when fasting, 100–128 when fed with sugar, 117–167 (an average increase of 40 per cent.) when fed with protein.[72]

Metabolism in rest and activity—Metabolism at a given temperature is generally much higher in the adult than in the larva, and higher in the larva than in the pupa: at 30° C. the pupae of flies consumed about 8 c.mm. per gm. per minute, the larvae about 33 c.mm., the imagines 97 c.mm. (Fig. 269).[6] This is the result mainly of differences in muscular activity. In one series of experiments on the bee, when this was at rest at 18° C., the oxygen consumption was 30 c.mm. per gm. per minute. This is equivalent to 1·8 litres per kg. per hour; and since we know that the bee consumes only carbohydrate

(p. 326), this will be equivalent to 9·09 calories per kg. per hour.* (The corresponding figure for the 'basal metabolism' in man is about 1·2 calories per kg. per hour.) During flight the oxygen uptake rose to 1,450 c.mm. per gm. per minute, a 48-fold increase.[94] Others have obtained a still higher rate of increase.[108] The Sphingid *Deilephila* in one experiment showed a 37-fold increase from 0·33 mg. of CO_2 per gm. per hour during rest, to 12·22 mg. during flight.[97] Various Lepidoptera at rest at 20–25° C. had an average oxygen consumption of about 8 c.mm. per gm. per min. During the violent vibration of the wings induced by nicotine poisoning, this increased some 200 times.[149]

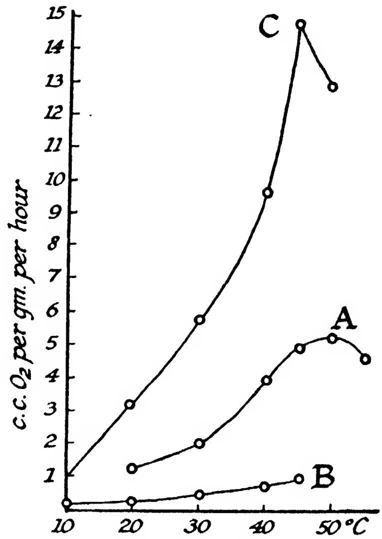


FIG. 269.—Temperature and respiratory exchange of flies in *A*, larval, *B*, pupal and *C*, adult stages (*after* BATELLI and STERN)

Ordinate: oxygen uptake in c.c. per gm. per hour. Abscissa: temperature in ° C.

Metabolism in relation to size—Since each species has its own characteristic intensity of metabolism, it is not possible to establish any law relating one with another; though among similar insects metabolism tends to be less intense in the larger forms.[60] But within a given species, at a given temperature, the metabolic rate shows a fairly definite relation with size. In general, metabolism per gram of body weight falls off as the insect grows, in such a way that respiration is proportional to the mass of the insect with a fractional exponent. This exponent is usually about 2/3 (0·67). Table 4 shows figures for *Melanoplus*.[14] Similar results have been obtained on *Locusta migratoria*.[28] In the three common cockroaches, the exponent varies from 0·75–0·8 instead of 0·67.[74] In *Blatta* the metabolic rate per gram is found to diminish as the insect grows from 80 to 200 mg., but thereafter it seems to remain constant[41]; and in larvae of *Tenebrio*, the rate is high until they reach 5 mg. weight, it falls rapidly to about 45 mg., and then remains roughly constant until they reach 140 mg.[135]

TABLE 4

Weight in gm.	*Ratio of CO_2 output per gm. of body weight.*	*Ratio of CO_2 output per gm. of body weight $^2/_3$.*
2·16	1,000	1,290
1·35	1,037	1,204
1·11	1,174	1,224
1·08	1,186	1,226
1·05	1,219	1,232
1·01	1,263	1,276
0·94	1,310	1,282

The exponent 0·67 is approximately the same as that which relates body weight with body surface. The two figures agree closely in *Locusta*[28]; in

* In the combustion of carbohydrate 1 litre of oxygen produces 5·047 calories.

Aphis rumicis the surface is estimated to vary with the weight to the power 0·60; in *Blatella germanica* 0·63.[165] The fall in metabolic rate with size is therefore sometimes referred to as the 'surface law'; but there is no known reason why the metabolism of cold-blooded animals should bear a relation to the body surface.

Effect of oxygen tension on metabolism—So far we have assumed that oxygen consumption is determined solely by the needs of the tissues. We must now consider under what conditions the supply of oxygen may become a limiting factor in oxidation. In general, oxygen uptake is more or less independent of the oxygen tension in the environment, within wide limits. In the mealworm larva the oxygen consumption begins to fall off when the tension drops below 10 per cent. O_2 in the air (Fig. 270, B)[181]; in the mealworm pupa at 20° C. it remains constant from 50 per cent. O_2 down to 5 per cent. O_2 (Fig. 270, A)[65]; in the termite, *Termopsis*, there is no falling off until a concentration of 2 per cent. O_2 is reached[35]; in the actively developing eggs of *Melanoplus*, concentrations between 21 per cent. and 3 per cent. O_2 have no effect, below 3 per cent. the oxygen uptake falls and,

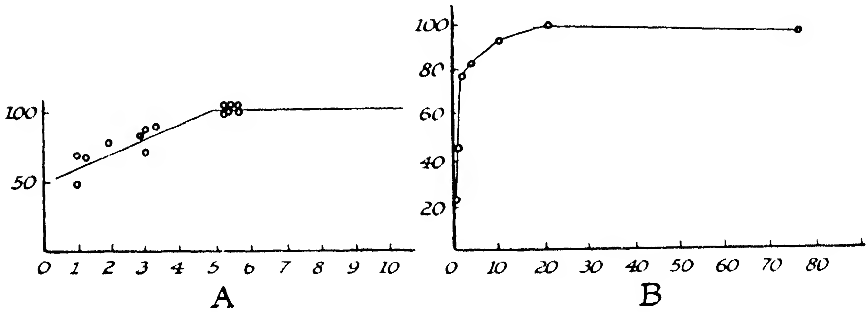


FIG. 270.—Relation between oxygen tension and oxygen uptake in pupa of *Tenebrio molitor* (*A*) (*after* GAARDER), and larva of *Tenebrio molitor* (*B*) (*after* THUNBERG)

Ordinates: oxygen uptake as percentage of uptake in air. Abscissae: oxygen concentration in volume per cent.

unlike most insects, at concentrations about 50 per cent. the respiration is stimulated.[18] The aquatic nymphs of Ephemeroptera show great differences in their ability to maintain their oxygen consumption with a falling oxygen tension. Certain species of *Baëtis*, accustomed to streams with abundant oxygen, are affected far more readily than *Cloëon dipterum*, which lives in ponds. But there is no strict relation of this sort with the type of habitat.[60]

The level of oxygen tension at which consumption is affected, must vary with the rate of metabolism. This can be seen in larvae of *Chironomus thummi*, in which the oxygen uptake of normal insects remained the same as in air, down to a concentration of 3 per cent. O_2; whereas in larvae which were recovering from exposure to nitrogen, and whose respiration was consequently increased 160 per cent. (p. 343), the oxygen uptake began to fall off below about 10 per cent. O_2.[76] Similarly, if an increase in temperature accelerates metabolism more than it favours the supply of oxygen by diffusion, the oxygen consumption will fall off sooner at a high temperature than at a low. Thus in the mealworm pupa at 20° C., oxygen uptake remains constant down to 5 per cent. O_2 in the air; at 32° C. it begins to fall off at about 10 per cent. O_2.[65]

Probably oxygen tension becomes a limiting factor only when the oxygen supply fails, and the tension in some part of the tissues becomes zero.[113] At 20° C. the oxygen tension in the tissues of the mealworm larva is about 16 per cent. of an atmosphere. Hence there is a gradient of 21 − 16 = 5 per cent. between the tissues and the outside air. The tension in the tissues would therefore be expected to fall to about zero when the oxygen in the air falls to about 5 per cent.; which agrees well with the experimental results (p. 342).[65] But it must be pointed out that this argument is to some extent invalidated by the regulatory mechanisms in the spiracles which have since been discovered (p. 189).

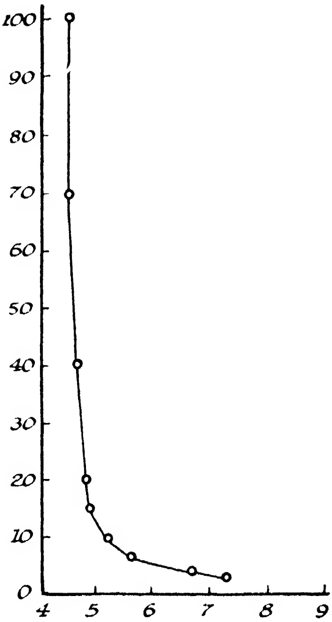


FIG. 271.—Effect of oxygen tension on the duration of the pupal stage of *Drosophila melanogaster* at 18–20° C.

Ordinate: oxygen per cent. (in hydrogen). Abscissa: duration of pupal stage in days (*after* KALMUS).

Oxygen tension may serve as a limiting factor in development. In the pupa of *Drosophila* the time required for development is prolonged from 4·8 to 7·2 days as the oxygen concentration falls from 20 per cent. to 3 per cent. The minimal concentration for development lies between 2–3 per cent.; and up to a concentration of 70 per cent. the rate is increased (Fig. 271).[98]

Anaerobic metabolism—If the mealworm is kept at a low oxygen tension, such as 3 per cent., for some hours, and then returned to air, its oxygen uptake is raised above the normal level for some time. This is attributed to the removal of intermediary products of metabolism which have been incompletely oxidized.[65] For the initial stages of energy metabolism, notably the production of lactic acid from sugar, take place anaerobically; the oxidation of such substances may be deferred until oxygen is restored, the insect accumulating an 'oxygen debt'. This process is well seen if the insect is kept in complete absence of oxygen. The cockroach *Blatta orientalis*, kept in nitrogen for ½ hour, contracts a debt which it requires 1½–2 hours to pay off; the extra oxygen consumed during recovery being just about equal to the volume it would have used during the time it was deprived of oxygen, and just about equal to that required for the complete oxidation of the lactic acid which has accumulated.[41, 167] Similar results have been obtained with the grasshoppers, *Melanoplus* and *Chortophaga* (Fig. 272)[16] and with *Chironomus* larvae.[76] In

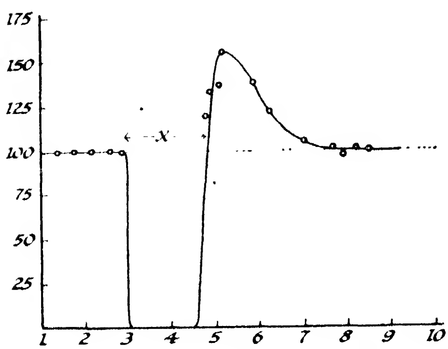


FIG. 272.—Curve showing the effect of immersion in water for 75 minutes on the rate of oxygen consumption in nymph of *Melanoplus differentialis* (*after* BODINE)

Ordinate: percentage oxygen consumption (taken as 100 per cent. before immersion). Abscissa: time in hours. X, period of immersion.

the grasshoppers, the blood increases in acidity from *p*H 6·8 to *p*H 5·8 [16]; and in the cockroach, and in *Chironomus*, this acid causes a liberation of carbon dioxide from bicarbonates in the blood and tissues.[76, 167]

The Oestrid larva *Gastrophilus* shows a remarkable capacity for living anaerobically; it has been kept for 17 days submerged in oil. But it shows almost no accumulation of lactic acid (0·4 mg. per gm. of live weight at the beginning, to 0·53 mg. at 14 days).[46] It seems to convert its store of glycogen into fat and to utilize the oxygen set free for energy production; glycogen disappears, and more fat is produced, in the absence of oxygen. Thus 100 gm. of larvae starved for 24 hours showed an increase of 0·03 gm. fat in the presence of oxygen, 0·06 gm. under anaerobic conditions.[103]

Respiratory quotient—When the oxidation of metabolites is complete the respiratory quotient (R.Q.), the ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed, gives some indication of the nature of the substances being utilized. For in the complete combustion of carbohydrate this ratio is 1·0, for fat and protein (when this is oxidized only as far as uric acid) it is about 0·7. Thus the R.Q. of the cockroach fed on starch is 1·01–1·07, on fat it is 0·78–0·83, and after starving for several days (when, as we have seen, fat is chiefly utilized) it falls to 0·65–0·85.[73] In termites the R.Q. is normally 1·0; it falls during starvation.[34] In the fasting mealworm it is 0·7.[133] In the bee, which, as we have seen, burns chiefly sugar, it is equal to 1·0 during rest, and during short flights.[94]

At very low oxygen tensions the R.Q. tends to rise, owing to the increasing acidity of the tissues driving off carbon dioxide; for example, in *Tenebrio* [181] and *Chironomus* larvae [76]; it may even go above unity, as in *Anax* larvae.[123] And during the process of recovery from oxygen want, as the carbon dioxide capacity of the blood and tissues increases again, the R.Q. falls for a time below the normal level.[167] Some Lepidoptera, such as *Agrotis segetum*, utilize sugar to synthesize the abundant fat in the egg yolk, and this transformation may send up the R.Q. to an average of 1·5–1·6 or a maximum of 2·09.[110] In insects exposed to low temperatures, the R.Q. may fall to a very low figure. In ants during the summer at 22° C. the R.Q. is 0·88, at 4° C. it is 0·50 [49]; in hibernating nymphs of *Chortophaga* at 27° C. the R.Q. is 0·83, at 15° C. it is 0·62.[106] The low temperature seems to cause some disturbance in metabolism, the nature of which is not known. The values at the low temperature are too low to be explained by an exclusive oxidation of fat, and too prolonged to be explained by the increased solubility of gases in the tissue fluids.

Metabolism during growth—In the egg, oxygen uptake runs parallel with visible development. In the silkworm, metabolism is active during the initial development up to the blastoderm stage; it then falls and remains at a low level throughout the latent period, to rise rapidly again when renewed development occurs in the spring.[120] In *Melanoplus*, respiration follows the same course, high in early development, low in diapause, and rising steeply during blastokinesis.[26] Whereas in *Chortophaga*, which develops without interruption, the oxygen uptake rises steadily until the time of hatching.[17] In *Melanoplus* the R.Q. is 1·0 during the first day of embryonic development; thereafter it is generally about 0·71, suggesting that fat is the chief normal source of energy [24]; but throughout development added glucose is readily oxidized by embryos isolated from the egg.[21] There are two periods in the

development of the *Melanoplus* egg, before diapause and when blastokinesis is complete, during which the R.Q. sinks very low, suggesting that fat is being utilized for the synthesis of oxygen-rich compounds—possibly the chitin of the serosal membrane (p. 2) and the pre-larval skin (p. 12) (Fig. 273).[24]

In the pupa, oxygen uptake follows a characteristic U-shaped curve; it is high immediately after pupation, falls to a minimum, rises again shortly

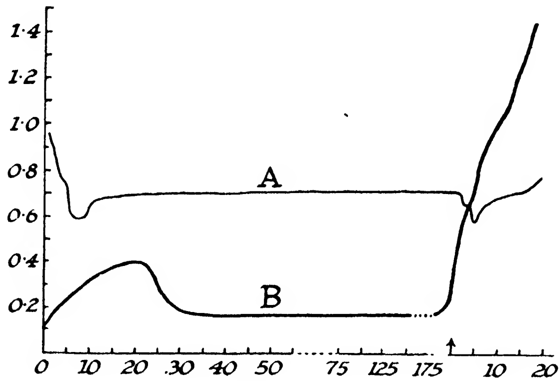


FIG. 273

A, respiratory quotient during entire embryonic development of *Melanoplus* (*after* BOELL). Ordinate: R.Q. Abscissa: days of development; pre-diapause period from 0–20 days; diapause from 21 days to the arrow; post-diapause period from the arrow to 17 or 19 days (hatching). *B*, relative oxygen consumption.

before emergence, and finally shows a slight depression before rising to the very high level associated with the escape of the adult insect.[174] Curves of this type (the final drop before emergence has not always been noticed) have been described in *Calliphora*,[63, 195] *Lucilia*[38] and other blow-flies,[174] *Drosophila*,[23] *Galleria* (Fig. 274),[39] *Ephestia*,[174] *Deilephila*,[85] and other Lepidoptera,[57] *Tenebrio*,[112] and other Coleoptera.[57] In the pupae of *Lymantria dispar*[4] and *Galleria*[176] the rate of heat production follows the same curve as that of oxygen uptake; but the calorific quotient, the ratio of the heat in gram calories to the oxygen uptake in millegrams, is very low (1·16–2·84 compared with a value of 3·3 for the combustion of fat), perhaps because of the large proportion of endothermic syntheses that are taking place.[4, 176]

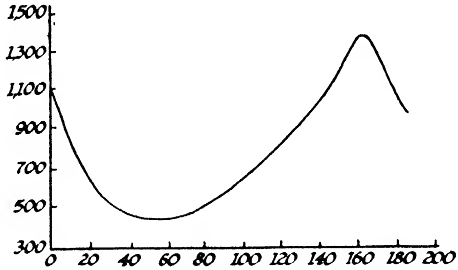


FIG. 274.—Oxygen uptake by pupae of *Galleria* at 30° C.

Ordinate: oxygen uptake in cubic millimetres per gram per hour. Abscissa: time in hours. (*After* TAYLOR and STEINBACH.)

The significance of this curve is not altogether clear. When there is a prolonged arrest of growth in the pupa, as in the overwintering pupae of *Deilephila*,[85] it is understandable that there should be a long period of low metabolic rate; but the same course is followed when development is apparently continuous.[85] It is certain that there is a real change in oxygen requirements: the fall is not due to failure in the oxygen supply; for in *Tenebrio* the oxygen tension in the tissues is actually greatest when the rate of metabolism is lowest.[65] In *Drosophila* the fall is attributed to changes in

the quantity or activity of the oxidizing enzyme system; i.e. this system is either destroyed in part and re-built, or temporarily inhibited by respiratory poisons [202]

It has often been suggested that the fall of the curve represents histolysis, the rise histogenesis; the intensity of metabolism at any moment being a measure of the amount of organized tissue present.[57, 112, 85] But this idea is not supported by histological observations [138]; in *Drosophila*, for example, histogenesis is finished before the curve of oxygen uptake reaches its minimum, and the adult organs are anatomically complete before half the pupal stage has elapsed, that is before the oxygen consumption begins to rise.[48]

The associated changes in the reaction of the blood are variable. In some insects the *p*H is said to fall, for example in *Attacus*,[51] and in *Leptinotarsa*, where it falls from 6·8 to 5·9 [57] as the respiratory rate decreases. In others, such as *Popillia*, there is no significant change [122]; while in *Galleria* the *p*H is highest (6·5) when metabolism if lowest, and falls to 5·8 when the respiratory rate increases again; here the *p*H is regarded as a reflection of the rate of carbon dioxide production.[175]

We have seen that the chief source of energy during pupal development is fat; and in some insects the respiratory quotient agrees with the expected value for fat combustion: 0·70 in *Tenebrio*,[112] 0·67–0·78 in *Drosophila*,[144] 0·69 in *Galleria*.[39] But in many cases, particularly during the middle period of pupal development, very low values are obtained: less than 0·7 in *Lucilia*,[38] 0·5–0·65 in *Calliphora*,[63, 195] 0·5 in *Attacus* (Lep.),[51] as low as 0·4 in *Popillia*,[121] and 0·42 in *Leptinotarsa*.[57] These values presumably indicate either the synthesis of constituents relatively rich in oxygen, or the temporary incomplete combustion of fats or other substances.

Metabolism during diapause and quiescence—Depression of metabolism is always a striking characteristic of arrested growth or diapause at whatever stage this may occur (p. 10, 67). In eggs of *Melanoplus* during diapause, respiration is maintained at about one-third or one-quarter of that shown by developing eggs at the same morphological stage.[17] The same depression is seen in overwintering pupae of *Deilephila*, in which it has even been suggested that metabolic rate is the causative factor distinguishing strains with and without a diapause.[85] The oxygen uptake by larvae of *Lucilia* increases from 0·34 c.c. per gm. per hour, to 1·10 c.c. when diapause is broken by suitable stimuli.[38] Pyralid larvae in diapause can withstand submergence in water for several days, whereas active larvae have not this resistance.[109] The summer wasp resting at 0° C. has an oxygen uptake nearly 20 times as great as that shown by the hibernating wasp at the same temperature,[137] and the ant *Formica ulkei* shows a similar reduction during hibernation.[49] We have already discussed the low values for the respiratory quotient which may occur during hibernation (0·6 in *Leptinotarsa*,[57] 0·62 in grasshopper nymphs,[106] 0·52 in ants [49]). These appear to indicate some change in metabolism, perhaps incomplete combustion, which is a direct effect of lowered temperature (p. 344).

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Chapter XIV

Water and Temperature

THE MOST important factors in the environment, which influence the physiology of insects, are temperature and humidity. In their effects, these are constantly reacting upon one another; it is therefore necessary always to consider them side by side.

WATER RELATIONS

Water content—The quantity of water in different insects ranges from less than 50 per cent. to more than 90 per cent. of the total body weight [21]; and there may be much variation within the same species, even when reared under identical conditions. The percentage tends naturally to be low in insects in which the cuticle contributes largely to the total weight; in the adult of *Calandra granaria* the water content is only 46–47 per cent. It is high in the thin-skinned larvae of Lepidoptera, &c.; thus in the large Saturniid caterpillar *Telea polyphemus* it amounts to 90–92 per cent.[21] The proportion of water is influenced to some extent by the quantity of fat present (p. 325); active larvae of *Phlebotomus papatasii* (Dipt.), in which fat composes 5 per cent. of the dry weight, contain 65–70 per cent. of water, hibernating larvae with 15 per cent. of the dry weight made up of fat, contain 52–56 per cent.[86] But here, as in other insects, the increase in fat will not explain the whole of the fall in water content; there is a second factor, a tendency to lose water before entering hibernation or diapause. Thus growing nymphs of *Chortophaga viridifasciata* may have a water content as high as 79 per cent.; on entering hibernation this falls to 65 per cent., rising again to 75 per cent. with the restoration of activity.[11] There is generally a fall in water content on pupation and a further fall in the imago; in *Popillia japonica* the larva contains 78–81 per cent. of water, the pupa 74 per cent., the adult, which has a much higher proportion of skeletal material, 66·6 per cent.[50, 53]

The ability to withstand a reduction in the water content varies considerably. Larvae of the beetles *Leptinotarsa*, *Popillia*,[50, 53] *Tenebrio*,[18] the caterpillar *Ephestia*,[83] and the bug *Rhodnius* [20] succumb when the water content falls from about 75 per cent. to about 60 per cent. or a little less. *Chortophaga* dies at 56–59 per cent.[51] The clothes-moth larva *Tineola*, with a normal water content of about 59 per cent., can survive with a content appreciably less.[55] The termite *Termopsis*, which normally has from 74–80 per cent. of water, dies as soon as this falls much below 68 per cent.[23]

Diminution in the water content usually depresses metabolism and retards development. If eggs of *Melanoplus differentialis* are dehydrated by immersion in hypertonic solutions, the oxygen uptake falls, more or less in proportion to

the concentration used.[12] Eggs of the weevil *Sitona* at 20° C. develop in $10\frac{1}{2}$ days in saturated air, in 21 days at a relative humidity of 62 per cent., below which they fail to develop altogether [2]; eggs of *Lucilia* at 22° C. develop in 23 hours at a saturation deficiency of 12 mm. of mercury, in a little over 20 hours at a saturation deficiency of 2 mm.[30]; and the duration of the pupal stage in *Popillia* increases as the water content becomes less.[50] The extreme cases are those already described in which eggs of some Collembola and grasshoppers remain dormant for months or even years in a desiccated state (p. 8). Sometimes the effect of desiccation is purely mechanical; the chorion of the egg may become too hard for the insect to break through (p. 1), or the fully developed insect in the pupa may lack sufficient volume of water in its blood to rupture the pupal sheath, as in *Lucilia*.[30]

Sometimes the rate of development is retarded at very high humidities; the pupal stage of *Lucilia* is prolonged by about 5 per cent. in saturated air [30]; pupae of *Sitotroga* (Lep.) develop in 12 days at a relative humidity of 22 per cent., in 17 days in saturated air at the same temperature [36]; and pupae of *Bruchus obtectus* (Col.) which require 14 days at a humidity of 45 per cent., require 22 days in saturated air.[36]

Evaporation—Water is lost by insects chiefly through evaporation. According to Dalton's law, the rate of evaporation from a water surface is proportional to the saturation deficiency of the air; as it is sometimes expressed

$$V = a.E(100 - H) + c$$

where V is the rate of evaporation, E is the aqueous vapour tension when the air is saturated at the temperature in question, H is the relative humidity and a and c are constants.[41] Dalton's law is applicable to the rate of loss of water from insects.[19] But there are many exceptions; and the physiology of evaporation is best appreciated by analysing the factors which cause departures from this rule. Some of these factors are physical, some physiological.

(i) One factor operative in still air is perhaps the increasing rate of diffusion as the temperature rises. At a given saturation deficiency, evaporation may therefore be expected to be greater at high temperatures than at low.[73] Thus in experiments of short duration, eggs of *Lucilia* lose more water in proportion to the saturation deficiency at 22° C. than at 14° C.[30]; and likewise in pupae of *Milionia* (Lep.) the evaporation through the integument per unit of saturation deficiency rises with rising temperature.[41]

(ii) High temperature may increase the permeability of the cuticle to water. That may be the explanation of the increasing loss in *Lucilia* eggs, and through the integument of *Milionia* pupae, with rising temperature, just noted. It is certainly the explanation in the cockroach. Other factors being equal, evaporation from the cockroach is proportional to saturation deficiency until the temperature reaches 30° C. There is then an abrupt increase in evaporation which is due to a change in the properties of the oily film that is responsible for the impermeability of the cuticle in this insect (p. 21).[73]

(iii) In connection with respiration we have already discussed some of the evidence which proves that the greater part of evaporation from insects takes place through the spiracles (p. 190). Further evidence to this effect has been obtained by sealing the spiracles with vaseline, or by leaving the spiracles open and covering the remainder of the body with vaseline, and comparing the

evaporation under these conditions. By such means it has been concluded that in the pupa of *Bombyx mori* the spiracles are responsible for about 66 per cent. of the total loss, and in the adult grasshopper *Gastrimargus* about 70 per cent., the loss through the cuticle taking place particularly at the intersegmental membranes.[41]

Most of the departures from Dalton's law to be observed in living insects are due to this fact that most of the water vapour escapes from the tracheal system (Fig. 275). Thus increasing the rate of flow of air over the cockroach has little effect upon evaporation from the body surface, but it causes a marked increase in evaporation from the tracheal system, probably by creating eddies within the tracheae.[73] The same thing has been observed in the pupae of *Milionia*.[41]

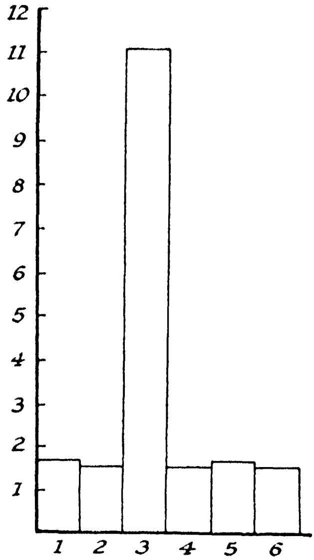

FIG. 275.—Daily loss of water in adult *Rhodnius* starved at 24° C., 0% Relative Humidity. During the third day the spiracles were kept open by exposure to 5% CO_2 (*after* WIGGLESWORTH and GILLETT)

Ordinate: loss of weight in mg. per day. Abscissa: days.

(iv) If the spiracles are opened more frequently than normal (p. 189), the rate of evaporation will be increased. Thus feeding, exercise, egg production, or any other factor which causes an increase in metabolism, will increase evaporation.[41] At 20° C., under constant conditions of air movement and saturation deficiency, the cockroach *Blatta* lost 3·9 mg. per hour by evaporation while at rest, 6·0 mg. per hour when stimulated.[73] Now increase in temperature is itself a factor which causes an increased ventilation of the tracheal system; a rise in temperature will therefore increase evaporation even when the saturation deficiency remains unchanged.[41]

(v) A departure from Dalton's law is also seen sometimes in very dry air. In the mealworm no effect of this kind can be discovered.[55] But in hibernating nymphs of *Chortophaga*, the rate of water loss falls off when the saturation deficiency exceeds 20 mm. of mercury.[11] In *Cimex*, also, the rate of loss becomes less than expected in dry air.[55] In the pupa of *Milionia* at 20° C. a similar break occurs at 11 mm. of mercury saturation deficiency.[41] Two explanations are offered for this phenomenon: (*a*) that an active regulation begins at very low humidities, the insect keeping its spiracles even more rigorously closed than usual [41]; (*b*) that in very dry air, water vapour escapes so rapidly from the tracheal system that the air in the tracheae becomes relatively dry, and the rate at which water will diffuse through the tracheal walls becomes the limiting factor in water loss; the level of dryness at which this occurs being peculiar to each species.[58]

(vi) Departures from the law may also be encountered at the other end of the humidity scale.[21] *Milionia* pupae lose less water than expected in very moist air, particularly at high temperatures.[41] The clothes-moth larva *Tineola*,[55] the mealworm larva,[55] and the grasshopper *Chortophaga* (Fig. 276) [51] actually increase their water content, they may even increase their total weight by 10 or 15 per cent., when starved at high humidity. This property, like the

absorption of water by hygroscopic substances, depends upon relative humidity, not saturation deficiency.[55] In *Chortophaga* this uptake ceases below about 82 per cent. relative humidity [51]; in *Tenebrio* below 88 per cent.[55] It can be observed in *Tenebrio* even when there is no possibility of the insect reingesting

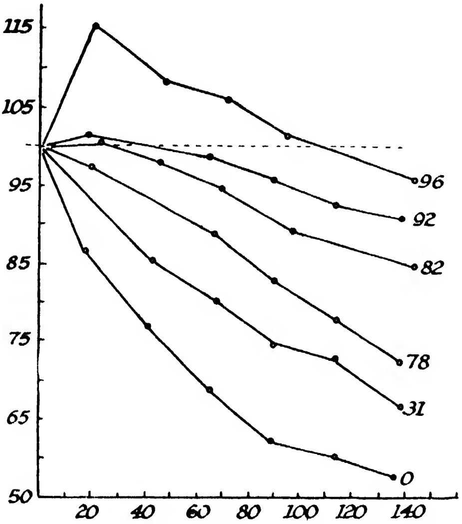

FIG. 276.—Rate of loss of weight in *Chortophaga viridifasciata* at the different relative humidities indicated at the end of each curve (*after* LUDWIG)

Ordinate: weight as percentage of original weight. Abscissa: time in hours.

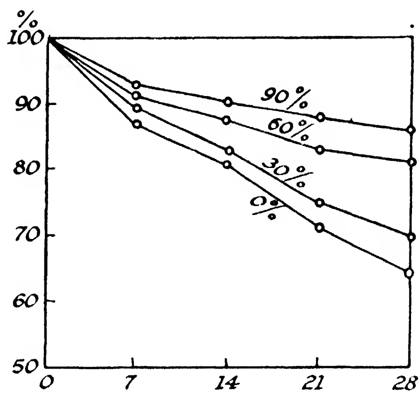

FIG. 277.—Effect of relative humidity on evaporation from the bed-bug *Cimex lectularius* at 8° C. (*after* MELLANBY)

Ordinate: percentage of original weight. Abscissa: days. Figures on the chart show the four relative humidities employed.

its hygroscopic excrement [93]; there seems in fact to be an active absorption of water, perhaps in the tracheal endings.[55] Not all insects are capable of this; it does not happen in *Cimex* (Fig. 277),[55] *Termopsis*,[23] or larvae of *Popillia*,[53] even in a saturated atmosphere.

Water balance—If the water content is to remain constant, the gain of water must equal the loss. *Gain of water* may take place: (i) with the food. This has already been discussed (p. 283); but a further example may be noted. If the bed-bug *Cimex* has been desiccated, it restores its normal body composition by retaining more water than usual at its next meal. It can therefore thrive in any atmosphere provided it is fed sufficiently often.[55] (ii) By absorption of liquid water through the cuticle (p. 21). (iii) By absorption of water vapour from the air as described in the last section. (iv) By the oxidation of hydrogen in the foodstuffs or reserves, and the retention of the so-called 'metabolic water'. Thus the complete combustion of 100 gm. of fat will yield 107 gm. of water. This is certainly an important source of water in insects such as the mealworm [18] or clothes-moth [3] living in very dry materials. It has even been suggested that such insects may increase their rate of metabolism during desiccation in order to make good their loss of water.[18] But it is doubtful if this mechanism could be of service; for increased metabolism necessitates increased respiration, which itself involves an increased loss of water.[59] *Loss of water* may take place: (i) by evaporation through the cuticle and the tracheal system as discussed in the last section. (ii) By the faeces;

insects which retain their excrement, or are efficient at extracting water from it before it is discharged (p. 300), are the most resistant to desiccation.

When the normal water balance is upset, it is usually in the direction of desiccation; the retention of water being one of the chief problems before all small terrestrial animals. The bed-bug *Cimex*, for example, always loses water unless exposed to an almost saturated atmosphere.[55] But in some insects adapted to very dry conditions, the water balance may be upset in the opposite direction. Thus the mealworm, which is able to maintain its water content unchanged even when starved for a month in absolutely dry air,[7, 18] is so well adapted to retain water, that if it is exposed to moderately moist air (relative humidity 70 per cent.), at a temperature of 30-37° C. at which the metabolic rate is high, it is unable to get rid of its water of metabolism, and hence the proportion of water in its body rises excessively.[55] The larva of the clothes-moth *Tineola* occupies an intermediate position; if starved in dry air its proportion of dry matter increases; if starved at a relative humidity of 90 per cent., its water content rises.[55]

Desiccation and survival—The length of time insects are able to survive under adverse conditions may be influenced by many factors, such as temperature, or starvation, as well as desiccation. Nymphs and adults of *Chortophaga viridifasciata*, at a given temperature, will survive 5-6½ days regardless of the humidity; here starvation is the cause of death.[51] Whereas survival of *Popillia* larvae varies with the humidity; they will survive less than 4 days at 0 per cent., 23 days at 82 per cent. humidity, death occurring when the water content is reduced from 81 per cent. to 55-59 per cent.[53] When all other factors save loss of water are excluded, the time of survival should be inversely proportional to the saturation deficiency; but since varying temperatures influence both the rate of metabolism, and the rate of water loss through the spiracles, this relation is seldom realized in practice.[58] Where the number of survivals among batches of developing eggs or pupae is being related with saturation deficiency, the duration of development, that is, the time of exposure at each set of conditions, must be taken into consideration. The true measure of evaporative power in such an experiment is the product of time × saturation deficiency. When this time factor is taken into account, it can be shown that the percentage of survivors among eggs of *Habrobracon* or pupating larvae of the flea is in fact inversely proportional to the saturation deficiency.[58] Now the time necessary for development falls off rapidly with rise of temperature at first, more slowly later (p. 368); while the saturation deficiency increases with temperature more rapidly as the temperature rises. Hence the product of time × saturation deficiency, first falls and then rises;

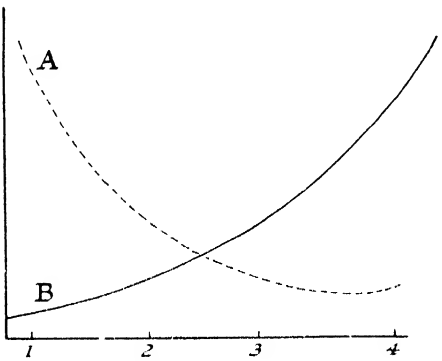

FIG. 278.—Chart to illustrate the effect of temperature on *A*, duration of development *B*, and saturation deficiency (*after* MELLANBY)

At temperature 1, development is stopped by cold, at 4 it is stopped by heat; the optimal zone of temperature is between 2 and 3, where the product of saturation deficiency and duration of development is lowest. (In many insects the relation is complicated by more frequent opening of the spiracles as the temperature rises.)

so that with a given water content in the air, the optimal conditions for development will be at some intermediate zone of temperature where this product is smallest (Fig. 278).[58]

TEMPERATURE RELATIONS

Heat balance and body temperature—In the insect at rest, over a moderate temperature range, heat production in metabolism balances heat loss, and the body temperature is the same as that of the surroundings.[33] In *Periplaneta* that is so between 10–22° C.,[63] in the Scarabaeid *Anomala expansa* between 15–30° C. and in the Libellulid *Orthetrum* between 12–23° C.[41]

Loss of heat is effected almost entirely through evaporation. In *Anomala*, and in the Psychid larva *Clania*, it is estimated that between 10–30° C. evaporation accounts for 80–100 per cent. of the total loss of heat, conduction and radiation for only 0–20 per cent.[41] It follows therefore that any factor which affects evaporation will influence the body temperature. At low temperatures, evaporation is depressed and the temperature of the body is often greater than that of the surroundings; at 5·5° C. the body temperature of the bee averages 4·7° C. above the surrounding air.[70] At high temperatures, evaporation is increased to a greater extent than heat production, and the body temperature falls below that of the air. In the cockroach this happens above 22° C.; it becomes increasingly evident above 32° C.[63] Air movement increases this cooling effect. The humidity of the air, also, greatly influences the body temperature through its effect on evaporation: except at low temperatures the body is always warmer in moist air than in dry [11]; in saturated air the body temperature of the cockroach is invariably above that of the surroundings.[63] Further, the cooling effect of evaporation is much more evident in live individuals, in which the tracheae are ventilated by respiratory movements. The Tenebrionid beetle *Adesmia*, alive on soil with a surface temperature of 38° C., had a body temperature of 36° C.; with the soil at 44° C. the body was at 39·5° C.; but in dead individuals the body was always at least 2° C. hotter than in the living.[17]

Gain of heat is chiefly the result of metabolism, particularly of muscular contraction. Gregarious larvae of *Vanessa* when clustered together may have their temperature raised $1\frac{1}{2}$–2° C. and this is sufficient to shorten the time of larval development by 1–3 days.[62] As was shown by Girard the body temperature of insects is raised above that of the air as soon as they become active; Hymenoptera, it was found, might show an increase of 4° C.; in Sphingids, the temperature in the thorax might be as much as 10° C. above that in the abdomen during vigorous flight.[33] A male of *Saturnia pyri* at an air temperature of 18° C. raised its thoracic temperature to 26° C. during fluttering.[4] Sphingids are said to be unable to take flight until their body temperature has been raised to 32–36° C. by preliminary fluttering, and during flight a temperature as high as 41·5° C. has been recorded in one insect.[28] Perhaps the thick covering of hairs is of some importance in preventing loss of this heat by conduction.[28]

Radiant energy from the sun is a source of heat in many insects; both visible light and infra-red rays will serve to heat the body above the surroundings.[29] The temperature of *Bombus* rose from 28·7° C. in the shade,

to 41·6° C. in the sun, in the course of five minutes, some of this increase being due to increased activity; and a passing cloud caused a rapid fall in temperature.[85] Many butterflies (*Erebia*, &c.) in high altitudes, are dependent on this source of heat, and are quite incapable of flight unless the sun is shining. The body colour influences the heat absorption; the dark brown race of the Acridiid *Calliptamus*, when exposed to the sun, was found to have a body temperature 4–5° C. higher than the buff race of the same species.[17] But the same colours which favour the uptake of heat during the day will favour the loss of heat by radiation during the night.[75]

The insect may control its temperature to some extent by changing its position in relation to the sun; locust hoppers in Palestine, with the body perpendicular to the sun's rays, had a body temperature of 41·7° C., with the body parallel to the sun's rays, 38·3° C.[31] The elytra of many beetles, and the wings of Lepidoptera, show a selective reflection for infra-red rays with a wave length of about 1μ, a region which is of high intensity in solar radiation.[76] This may afford some protection against excessive heating by the sun; and the subelytral air space of beetles is a considerable protection [48]; on the other hand, metallic and interference colours appear to have no influence on the absorption of heat.[29]

Temperature regulation in Hymenoptera—It is evident that in many insects the fluctuations of body temperature are subject to some degree of regulation.[10] A much more definite regulation exists among the social Hymenoptera.[38] Among ants this consists only in opening and closing the entrance to the nest according to the temperature, and in selecting the site for the nest—under stones or with a cap of earth to collect and retain the heat of the sun.[84] The wasp *Polistes* likewise obtains the heat necessary for its brood by solar radiation as well as from the air; but if the temperature becomes excessive, it cools the nest by fanning with its wings (at 31·5–35° C.), and often combines this with bringing water and applying it to the comb (at 34–37·5° C.).[84] *Vespa* also brings water to cool the nest by evaporation, or it may use fluid which it causes the larvae to emit.[92] In *Apis*, the hive is maintained during the summer at a temperature around 34–35° C. If it rises above this it is cooled by fanning and perhaps by the carriage of water. During the winter, the temperature of the hive goes through a series of cycles (Lammert's cycles) each lasting about 22–23 hours. The temperature falls to a minimum of 13° C., rises rapidly to a maximum of 24–25° C. and falls very gradually again to 13° C. The bees on the surface of the cluster are stimulated to muscular activity by a temperature of 13° C.; activity ceases again when the temperature has risen. It is estimated that in a hive of average size, 20 gm. of sugar, yielding 80 calories, may be consumed during one Lammert's cycle; if the outside temperature is unduly low the bees draw excessively upon their winter stores.[81]

Resistance to high temperature—The resistance of insects to high temperatures is complicated by the interaction of other factors. In nature insects may rest in spots cooled by evaporation, and are thus able to exist in environments far above the lethal temperature; even in laboratory experiments this habit may give an entirely false impression of their resistance.[56] The humidity of the air has a great effect on resistance: the cockroach *Periplaneta* dies at 38° C. in moist air; in dry air it can survive at a temperature of 48° C., being able to cool itself for a time by evaporation.[63] But this ability exists

only in insects above a certain size. For the rate at which heat is taken up by the body is proportional to the surface *area*, whereas heat loss, being brought about as we have seen by evaporation, is proportional to the *volume* of water evaporated. As the insect becomes smaller, the ratio of volume to surface becomes less, and below a certain size it could only lower its body temperature appreciably by evaporating more of its water than it could afford to lose.[54] Thus in such small insects as the louse *Pediculus*, the flea *Xenopsylla*, or the blow-fly *Lucilia*, the humidity of the air makes no difference to the lethal temperature when the duration of exposure does not exceed one hour. In larvae of *Tenebrio*, humidity has no influence in the case of small individuals; these die just below 42° C. at all humidities; but larvae weighing over 100 mg. can resist 43° C. in dry air, since they are able to cool themselves to some extent (Fig. 279).[54]

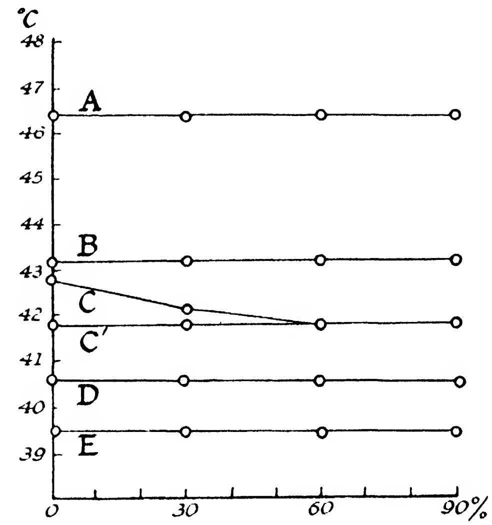

FIG. 279.—Highest temperatures at which certain insects can survive exposure for 1 hour with air of different humidities (*after* MELLANBY)

Ordinate: temperature in ° C. Abscissa: relative humidity. *A*, *Pediculus humanus*; *B*, *Lucilia sericata*; *C*, *Tenebrio molitor* larvae over 100 mg. weight; *C′*, the same, under 30 mg. weight; *D*, *Xenopsylla cheopis* adults; *E*, *X. cheopis* larvae.

In experiments of a longer duration, such as 24 hours, humidity may have the opposite effect; the greater evaporation in dry air may result in the insect dying from desiccation; therefore in long exposures it may withstand a higher temperature in moist air than in dry: *Blatta orientalis* withstands 37–39° C. for 24 hours in moist air, 34–36° C. in dry air.[35] If the insect retains its water efficiently, this effect is not apparent: in experiments lasting 24 hours mealworm larvae can resist 38·5° C. at 90 per cent. and 0 per cent. relative humidity; adults of *Xenopsylla* can resist 38° C. at both humidities; whereas the louse *Pediculus* can withstand 38° C. at 90 per cent., 33° C. at 0 per cent., *Lucilia* 36° C. and 32° C., larvae of *Xenopsylla*, which are quite unable to resist desiccation, 36° C. and 22° C. (Fig. 280).[54] The combined effects of temperature and humidity in affecting survival may be illustrated in the form of a 'thermohygrogram of mortality' as shown in Fig. 281.[1, 42]

Increase in temperature also stimulates metabolism; consequently, in some insects, exhaustion of the food reserves may be the real cause of death at high temperature. In nymphs of *Pediculus* the state of nutrition makes no difference to the lethal temperature in 1 hour experiments, all die at 46·5° C.; but in 24 hour experiments the unfed nymphs die at a lower temperature than those which have been fed.[57] The same is seen in *Trialeurodes*.[91]

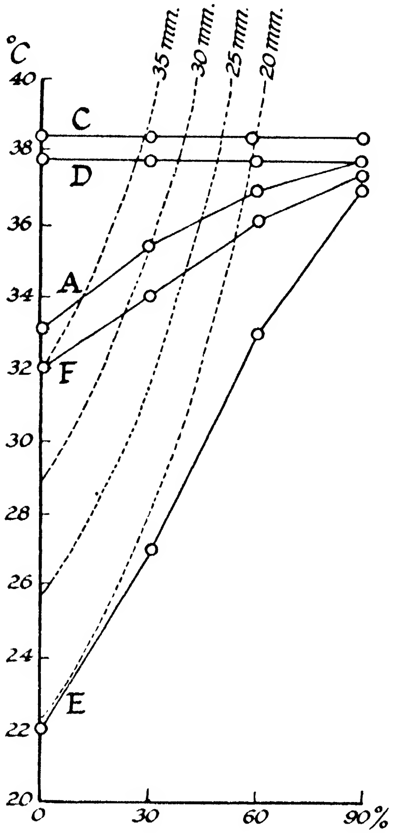

FIG. 280.—Highest temperatures at which certain insects can survive exposure for 24 hours with air of different humidities (*after* MELLANBY)

Ordinate: temperature in ° C. Abscissa: relative humidity. Lines of equal saturation deficiency are also drawn. Lettering as in Fig. 279. A, *Pediculus*; C, *Tenebrio* larvae; D, *Xenopsylla* adults; E, *Xenopsylla* larvae; F, *Anopheles* adults.

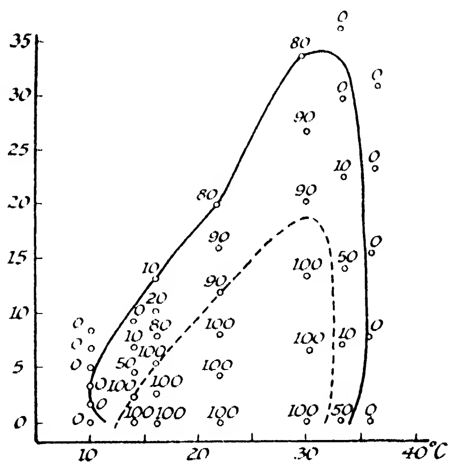

FIG. 281.—Mortality limits of pupae of *Lucilia sericata* at different combinations of temperature and humidity (*after* EVANS)

Ordinate: saturation deficiency in mm. of mercury. Abscissa: temperature in ° C. Figures represent the percentage emergence. The broken line encloses the region of 100% emergence, the continuous line that of 100% development.

Thermal death points—The actual temperatures which the tissues can withstand when these complicating factors have been excluded vary considerably from one species to another. Chironomid larvae have been found breeding in the water of hot springs with a temperature of 49–51° C.[15]; the larva of the cheese-fly *Piophila casei* will resist 52° C. for 1 hour.[82] In 1 hour experiments the thermal death point of *Pediculus* is 46·5° C., *Lucilia* 43° C., *Xenopsylla* adults 40·5° C. and larvae 39·5° C.[54] Heat stupor, which is the prelude to death, occurs in the adult flies of *Musca* from 45–46·5° C., in *Stomoxys* at 43·5° C., in *Fannia* at 39–41° C.[65]

Preferred temperature—In spite of the fact that insects are poikilothermic, their body temperature normally following pretty closely that of the environment, they are to a great extent orientated by temperature. When offered a

wide range of temperatures, young feeding larvae of *Musca* show a 'thermo-preferendum' of 30–37° C. (which is partly a direct response to temperature, partly a response to products of the fermentation which this temperature has induced in the food medium); by the 6th day of larval life, when they are full grown, these larvae all congregate below 15° C. Larvae of other dung breeding flies have a preferred temperature which accords closely with that of their normal breeding places: *Lyperosia* 27–33° C., *Stomoxys* 23–30° C., *Haematobia* 15–26° C.[87]

This choice of temperature may be greatly influenced by the conditions to which the insect has been previously exposed: the ant *Formica* showed a preferred temperature of 23–24° C. after exposure to 3–4° C.; but congregated chiefly between 31–32° C. when accustomed to a temperature of 25–27° C.[37] On the other hand, hungry females of the mosquito *Culex fatigans*, when offered two alternative temperatures, always avoid the higher, even down to 15° C. or lower.[88]

At low temperatures, the exact level being different for each species, the insect comes to rest and shows no spontaneous activity. As the temperature is raised it becomes normally active, then excessively active, and ultimately it passes into a state of heat stupor followed by death. The physiological differences between species were clearly seen among the insects on a sand dune, the order of their activities during the day being determined largely by the procession of temperature [22]: normal activity began in *Geopinus* at 7° C., *Melanoplus* 10° C., *Sphex* 17° C., *Cicindela* 18° C., *Chlorion*, *Gryllus* 20° C., *Bembex* 26° C., *Microbembex* 30° C.[90] The temperature at which activity begins, and the preferred temperature, seem to bear some relation with the thermal death point. We saw in the last section that the thermal death point in *Musca* was higher than in *Stomoxys*, which was higher than in *Fannia*. The preferred temperature shows the same sequence: *Musca* 33·4° C., *Stomoxys* 27·9° C., *Fannia* 23·7° C.[65] It is interesting to note that *Musca* and *Stomoxys* adults stand in the same relation as do their larvae.[87] But here again, the temperature required for a given level of activity is by no means fixed for a given species: in blow-flies, for example, the effect of any temperature is determined largely by the conditions to which the insect has just been exposed.[64]

The effect of atmospheric humidity on the preferred temperature is very difficult to test, because it is impossible to arrange a gradient of temperatures without at the same time creating a gradient in relative humidity. But in *Formica rufa*,[37] in certain beetles,[9] and in *Blatta*,[34] it is said that air humidity does not influence the preferred temperature. Some insects have the same preferred temperature irrespective of their previous treatment, but others collect chiefly at a lower temperature if they have been partially desiccated by exposure to dry conditions. The beetle *Adesmia clathrata* preferred 39·4° C. when in moist conditions, 36·6° C. after keeping in drier air.[9] Cockroaches kept in a graded temperature apparatus tend to settle down at a progressively lower temperature as time goes on, especially in dry air.[34] *Blatta* normally shows a preferred temperature or 'indifference zone' at 20–29° C.; individuals desiccated to 70 per cent. of their original weight had this zone shifted to 12–23° C., but returned to the normal range after drinking (Fig. 282).[34]

Resistance to low temperature—According to their resistance to cold, insects fall into three groups.

(i) Those accustomed to warm surroundings, like many tropical species or many of the insects that infest stored products, soon die even at temperatures well above freezing-point. The cause of death is not understood. It is often attributed to the accumulation of toxic products which at normal temperatures would be eliminated. In the honey-bee, which dies very quickly at temperatures between 1° and 8° C., death is attributed to a differential effect of cold on the various steps in the utilization of sugar: at 1° C. metabolism in the tissues still goes on pretty actively (in Pyralid larvae oxygen consumption continues down to − 12° C.[44]) but absorption from the gut is arrested; and since the bee is dependent on sugar in the food it soon dies of starvation at low temperature.[40] This type of effect by cold is sometimes termed the 'quantity factor' because it must act for some time before it causes death.[68]

(ii) Most insects are killed as soon as their tissues freeze. Since this effect

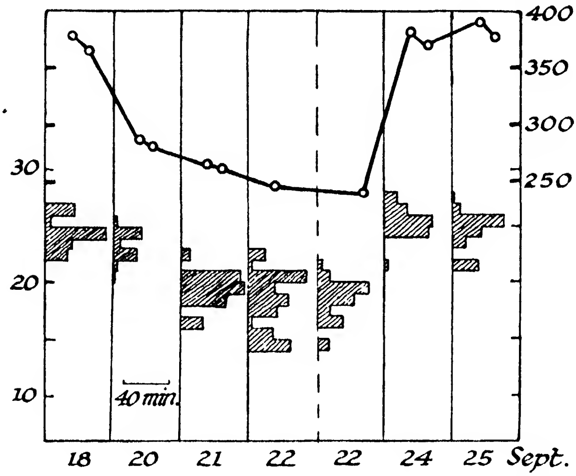

FIG. 282.—The curve shows fall in weight of a cockroach *Blatta orientalis* during desiccation and rise in weight again after drinking (on Sept. 22nd).

Ordinate (to the right): weight in mg. Abscissa: day of month. The blocked figures were arrived at by adding up the periods longer than 2 minutes during which the insect remained at a given temperature. Ordinate (to the left): temperature in ° C. The whole chart shows the fall in the preferred temperature on desiccation, and the return to normal following drinking. (*After* GUNN.)

depends only on the level of temperature it is sometimes termed the 'intensity factor' in cold resistance.[68] The actual cause of death from freezing is uncertain. It is attributed sometimes to dehydration of the tissues, sometimes to mechanical injury by ice crystals [78]; it is well known that many oxidation systems in cells are dependent on the integrity of cell structure and are destroyed by freezing.

(iii) A few insects can withstand complete freezing; but they die from some unknown cause when the temperature is lowered still further.[78]

Supercooling—'Cold hardiness' in insects is chiefly a matter of prevention of freezing. When the temperature is lowered, the water in the insect's body behaves like water in capillary tubing; it becomes supercooled, and ice does not form until the temperature has fallen far below the true freezing-point. The temperature of the insect will therefore follow that of the air until it has fallen perhaps to − 10° or − 15° C. (the 'critical point' or 'undercooling temperature'); it then suddenly jumps up to say − 1·5° C. through

the liberation of latent heat, and proceeds to fall once more. It is this 'critical point', where freezing begins, which varies in different species (Fig. 283).[4]

Supercooling is usually exaggerated in hibernating insects, some of which have been said to withstand − 50° C.[90] The increased resistance is generally associated with loss of water.[77] Nymphs of *Chortophaga* respond to cold by decreasing their water content from 79 per cent. to 65 per cent. and maintain this low level during hibernation.[11] In the beetle *Popillia* the undercooling temperature and the water content both fall during the winter when the cold hardiness increases greatly; and during the summer this insect may be 'artificially' hardened by desiccation: beetles dehydrated experimentally to half their body weight can resist a temperature of − 28° C.[68] The blow-fly larva *Lucilia* can resist only − 2° C. when actively feeding, − 10° C. in the pre-pupal stage when it has lost much of its water.[24] It is uncertain whether the water eliminated at low temperatures is lost by the excretory or the respiratory systems; but in the case of *Leptinotarsa* there seems to be some connexion between this loss and the general mechanisms of water retention. Thus strains of *Leptinotarsa* adapted to life in Arizona are far more efficient at

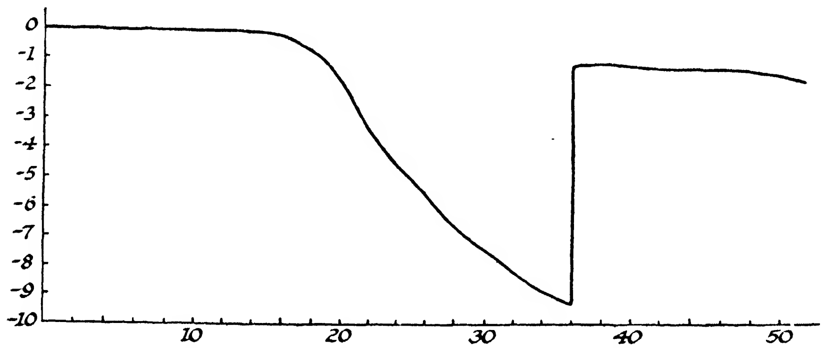

FIG. 283.—Body temperature of a *Saturnia pyri* male exposed to an air temperature of − 13·5° C., showing supercooling (*after* BACHMETJEW, 1899)
Ordinate: temperature in ° C. Abscissa: time in minutes.

retaining water under dry conditions than the Chicago strain; but they die off for the most part during the winter when brought to the Northern States, since they fail to eliminate their water before hibernation.[89]

But the capacity for supercooling is not necessarily associated with water loss. Full grown larvae of *Ephestia*, feeding, in early prepupal stage, and in the cocoon, have almost the same percentage of water; yet their undercooling points are − 5·8° C., − 8·0° C. and − 21·3° C. respectively. Adult bugs, *Leptocoris*, kept over calcium chloride until they had lost 20 per cent. of their weight, showed no change in their undercooling point. And hibernating bugs, *Chlorochroa*, after feeding for 5 days, increased their water content from 53 per cent. to 67 per cent., but their undercooling point remained stationary. Newly hatched larvae of *Ephestia*, *Sitotroga*, &c. have an undercooling point of about − 27° C.; after their first feed it is raised to − 6° C.[78]

The nature of the change which increases the capacity for supercooling is uncertain. This capacity is diminished by injury; for example, in *Bruchus* the undercooling point is normally − 15 to − 20° C.; if the beetle is pierced it is only − 8 to − 10° C.[78]; and after repeated freezing and thawing, super-

cooling is eliminated altogether, and freezing takes place as soon as the freezing-point of the body fluids is reached.[4] It has been shown that ice crystals forming in solutions rich in hydrophilic colloids are liable to become covered with

FIG. 284.—Metabolism of the bee at different temperatures (*after* PARHON)

Ordinate: oxygen uptake in litres per kg. per hour. Abscissa: temperature in ° C.

a sheath of dehydrated colloid, and thus fail to 'seed' the entire solution [78]; the quantity of such colloids may be a factor in supercooling. It has been suggested that the water which remains unfrozen at − 20° C. is 'bound' to the tissue proteins [74]; but there is at present no way of distinguishing 'bound' water from water which is supercooled from some other cause.

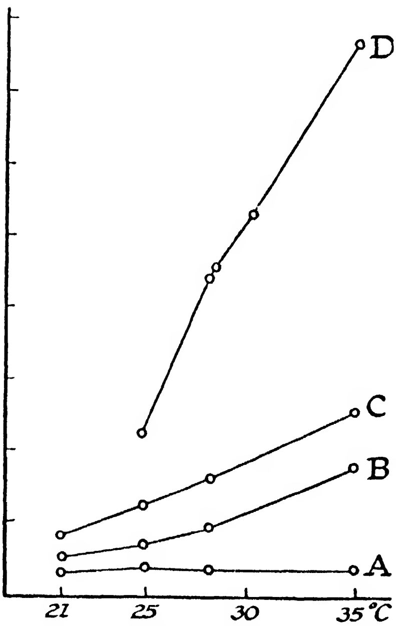

FIG. 285.—Effect of temperature on oxygen consumption in the mud-dauber wasp, *Sceliphron caementarium*, at different stages of development (*after* BODINE and EVANS)

Ordinate: relative oxygen consumption. Abscissa: temperature in ° C. *A*, larva in diapause. *B*, larva coming out of diapause. *C*, pupa. *D*, pupa ready to emerge.

Effects of temperature on metabolism and growth—As was first pointed out by Pflüger, whereas the metabolism of warm-blooded animals is depressed as the external temperature rises, the metabolism of cold-blooded animals increases. The increased activity which the insect shows with rising temperature accounts for most of this increase. What happens to the extra energy produced in the resting or narcotized animal as the result of raising the temperature is not known, but much of it must be expended by the augmented movements of the internal organs. In the developmental stages the extra energy is expended on growth, which is correspondingly accelerated.

Complicating factors are met with in the honey-bee, which increases its metabolism as the temperature falls in order to maintain the temperature of the hive (p. 360). Its rate of metabolism shows a maximum at 10° C. and then falls to 0° C. (Fig. 284).[43, 66] Diapause also may obscure the relation between temperature and metabolism: the oxygen uptake in eggs of *Samia cecropia* is little affected by raising the temperature from 16° C. to 35° C. during the

'incubation period', but is greatly stimulated during active development[60]; and in the mud-dauber wasp *Sceliphron*, the quantitative response to temperature, which is very slight during diapause, becomes very marked when diapause is broken (Fig. 285).[13] It is possible, also, that there may be some degree of acclimatization to the altered temperature; *Aeschna* nymphs show an increase in oxygen consumption at high temperature and a decrease at low, but after a day or so under either condition metabolism tends to return to the same intermediate level.[80]

Optimum temperature—Each species has a wide range of temperature between cold stupor and heat stupor within which it can perform its normal functions. Towards the limits of this range, growth or reproduction or some other function may be adversely affected, and some point in the middle of the range is sometimes described as the optimum temperature.[65, 90] This point is often difficult to define, and it is evident from what has been written in earlier sections of this chapter that the optimum may vary with the humidity.

A more definite conception of an optimum temperature is possible in considering development of the egg or pupa. Table 5 shows the effect of temperature

TABLE 5

Temperature	*Duration of pupal stage*	*CO_2 produced in total pupal life in litres per Kg. of pupa*	*CO_2 in cc. per Kg. of pupa per hour*
32·7 . . .	139·9	59·3	427
27·25 . . .	172·5	58·0	336
23·65 . . .	234·1	59·1	252
20·9 . . .	320	59·6	186

on pupal development in *Tenebrio*. As the temperature is raised from 21° C. to 33° C., the duration of pupal life is shortened from 320 hours to 139·9 hours,

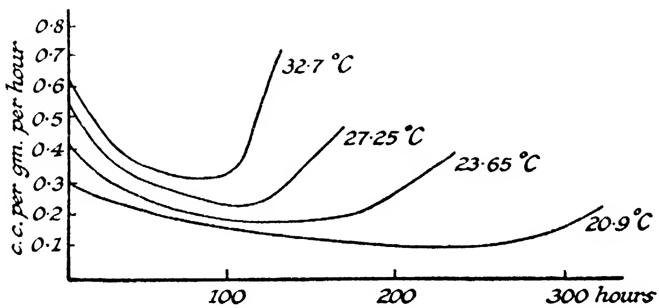

FIG. 286.—Respiratory exchange in pupae of *Tenebrio molitor* kept at four different temperatures throughout the pupal stage (*from* GAARDER *after* KROGH)

Ordinate: CO_2 production in c.c. per gm. per hour. Abscissa: time in hours.

but the total carbon dioxide production is unchanged (Fig. 286). There is no optimum temperature at which metabolism is smallest, and the efficiency of growth therefore greatest.[46] The same applies to the eggs of the beetle *Acilius*,[47] and in the pupa of *Drosophila* between 14° C. and 25° C.[27] But this is not

true of all insects. At abnormal temperatures the pupae of various Lepidoptera (*Agrotis*, *Loxostege*, *Ephestia*) show a higher oxygen uptake, more fat consumption, great pupal mortality and shorter imaginal life ; the temperature at which oxygen uptake is smallest may be regarded as the optimum.[45] In the pupa of *Galleria* the total metabolism is lowest at 30° C. and increases above and below this temperature ; the respiratory quotient being constant at 0·69 throughout.[25] Alternatively the optimum temperature may be regarded as that at which the greatest percentage of individuals complete their development ; or that at which the time of development is shortest,[8, 32] in other words, where the temperature velocity curve (Fig. 287, A, *b*) has its peak.

Mathematical descriptions of temperature effects—Fig. 287 A, *a*, shows the relation of temperature to the duration of egg development in the weevil

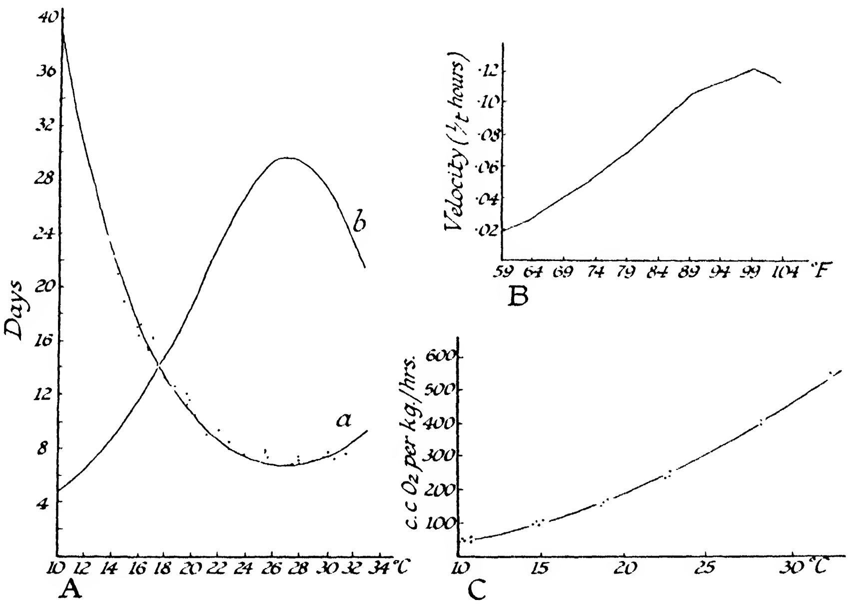

FIG. 287.—Effect of temperature on physiological processes

A, curve a, relation between temperature and duration of egg development in *Sitona lineata* ; curve b is the reciprocal of this curve and represents velocity of development (*after* ANDERSEN). B, relation between temperature and velocity of pupal development in *Phormia regina* (*after* MELVIN). C, relation between temperature and rate of oxygen uptake in pupa of *Tenebrio molitor* (*after* KROGH).

Sitona, and Fig. 287, A, *b*, shows its relation to the reciprocal of duration which indicates average rate of development.[2] Fig. 287, B, shows the rate of development in eggs of *Phormia* (Dipt.) plotted against temperature,[61] and Fig. 287, C, oxygen consumption in the pupa of *Tenebrio* against temperature.[46] In all cases the curve of intensity or velocity is S-shaped. It rises slowly at first, then steeply, then more slowly to a maximum and falls again to end abruptly at the lethal temperature. Sometimes, as in Fig. 287, C, during the steep rise it approximates to a straight line.

This curve represents the sum or resultant of an immense number of chemical and physical reactions, many of which must be differently affected by changes of temperature.[72] But in spite of this, numerous attempts have been made to

describe these curves by simple equations. Such formulae can be made to fit particular cases, and to that extent they have some descriptive value, but none of them is of sufficiently general application to be regarded as embodying any rational principle.[6] In view of the vast amount which has been written on this subject we must consider the various formulae that have been proposed.

(i) *That the velocity is proportional to the temperature.*

$$v = k(t - a)$$

where v = velocity, t = temperature, k and a = constants.

Or

$$v_{(t+10)} = v_t + K_{10}$$

where K_{10} is the increase in velocity for a rise of temperature of 10° C. from t to $t + 10$.

This relation holds very well over the normal range of temperature at which the egg of *Acilius* develops,[46] and for the pupal development of *Tenebrio*

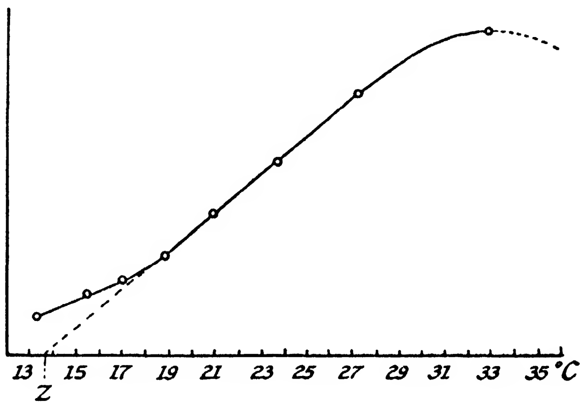

FIG. 288.—Relation between temperature (abscissa) and velocity of development (ordinate) in pupae of *Tenebrio* (*from* IMMS *after* KROGH)

The temperature Z, at which the straight part of the velocity curve meets the temperature axis when extended, represents the theoretical 'developmental zero'.

between the temperatures 18·5–28° C., but beyond these temperatures (normal development in *Tenebrio* occurs from 13·5–33° C.) the curve is not straight but bends upwards at lower temperatures and downwards at higher (Fig. 288).[46] With these same departures at the upper and lower limits, this linear relation between rate and temperature holds for the larval and pupal development of many Lepidoptera and Diptera,[69] and the egg and pupal development in *Popillia*.[49] It holds, also, for the rate of metabolism of *Dixippus* over most of the range from 5–35° C., provided it remains at rest, and for the respiration of various pupae.[16]

Thermal summation—In the first equation above, the constant a represents the temperature at which the straight line, when prolonged, meets the temperature axis. This temperature is termed the 'developmental zero'. It is the temperature at which development would cease if the curve did not in fact turn upward in this region.

If y is the time required for complete development at temperature t, the equation may be written

$$y(t - a) = K$$

in other words, the product of time of development in days × the excess of temperature above the developmental zero in °C. is constant. This value K in 'degree-days' is termed the 'thermal constant'. Thus, where the linear relation between velocity and temperature holds, each developmental process will have a characteristic thermal constant and will require a fixed number of 'degree-days' to bring it to completion. Therefore, even if the temperature is changed in the course of development, it is theoretically possible to predict the time necessary for its completion by adding up the number of 'degree-days' contributed at each temperature. This procedure is called 'thermal summation'.[8, 69] Apart from the linear relation between velocity and temperature, it involves the further assumption that the accelerating effect of temperature is the same at all stages in the developmental process. Where the relation between rate of development and temperature is not linear, it is possible to apply this summation procedure only after a fairly complete curve has been obtained experimentally, since the thermal constant changes its value at each temperature.[79]

Even where the velocity curve is linear over most of its course, it becomes less steep as it approaches the lowest temperatures; so that the 'developmental zero' is not in fact the true threshold of development; the temperature at which development ceases lies appreciably lower.[69] Thus in *Lucilia* the 'developmental zero' is at 12° C., the lower limit of development at 9–10° C.[24]; in *Rhodnius* these temperatures are 10° C. and 6° C. respectively.[32]

Another disturbing element in the summation of temperatures is the fact that in some cases an alternation of temperatures seems to stimulate development. Thus eggs of *Melanoplus atlanis* collected in the field and exposed at a constant temperature of 32° C. developed in 5 days; if they were exposed each day for 16 hours at 12° C. and 8 hours at 32° C., they developed in 3 days.[67] In the eggs of *Drosophila*, there seems to be no true stimulation of this kind; but development may be more rapid than anticipated from the thermal summation owing to the temperature-velocity curve deviating from the straight line at the lower temperatures.[52]

(ii) *That the logarithm of the velocity is proportional to the temperature.*

$$v = AK^t$$

where v = velocity, t = temperature and K and A = constants. Or

$$v_{(t+10)} = v_t Q_{10}$$

where v_t = velocity at temperature t, $v_{(t+10)}$ = velocity at temperature 10° C. higher, and Q_{10} = the temperature coefficient. Or

$$Q_{10} = \left(\frac{V_1}{V_2}\right)^{\frac{10}{t_1 - t_2}}$$

where V_1 and V_2 are the velocities at temperatures t_1 and t_2.

This is the Q_{10} rule of van't Hoff and Arrhenius, which holds fairly well for chemical reactions. In these the value of Q_{10} is usually between 2 and 3, but even in inorganic reactions some variation in Q_{10} always occurs. In biological reactions the value of Q_{10} usually decreases progressively as the

temperature increases. In the development of *Tenebrio* pupae, Q_{10} has the following values [46]:

10–15° C.	5·7
15–20	3·3
20–25	2·6
25–27·5	2·3
27·5–30	2·1
30–32·5	2·0

Therefore this rule rarely holds except over a very limited temperature range.

(iii) *That the logarithm of the velocity is proportional to the reciprocal of the absolute temperature.*

$$v = a.k^{\frac{1}{T}}$$

where v = velocity, T = the absolute temperature and k = a constant. Or

$$V_2 = V_1 e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where V_1 and V_2 are velocities at the absolute temperatures T_1 and T_2 respectively, e is the base of natural logarithms, and μ a constant which is termed the 'thermal increment' or 'temperature characteristic'.

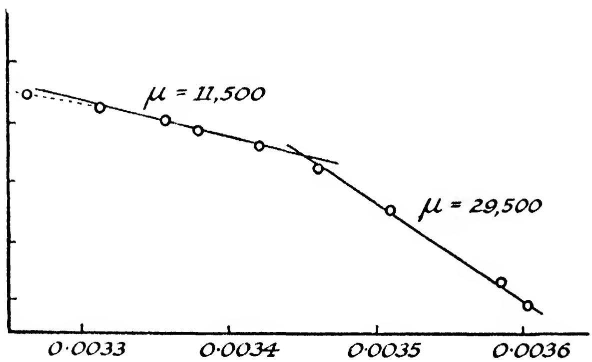

FIG. 289.—Oxygen consumption in *Dixippus* at different temperatures (data of v. BUDDENBROCK and ROHR as plotted by CROZIER and STEIR)

Ordinate: logarithm of oxygen consumption in c.c. per hour. Abscissa: reciprocal of the absolute temperature.

This is the law of Arrhenius, which seems to hold good for chemical processes with more accuracy than the Q_{10} rule. But over the narrow range of temperature at which living organisms exist, the reciprocal of the absolute temperature is practically a linear function of the ordinary temperature, so that in biology there is no practical difference between μ and Q_{10}.[6]

When the logarithm of the velocity is plotted against $\frac{1}{T}$ the slope of the curve is determined by the value of μ. In some of the processes of insect physiology a continuous straight line is obtained. But more often the slope changes. It is sometimes claimed that when this happens the change is always abrupt; in other words, that the value of μ is suddenly altered at a given temperature (Fig. 289). The theoretical explanation given, is that over each range of temperature one particular chemical or physical reaction is the 'master

reaction ' or ' limiting factor ', which determines the rate at which the process as a whole can proceed, and that each successive master reaction has a different ' temperature characteristic '.[26] This conclusion rests upon the inspection of the curves obtained experimentally, and some authors consider that the value of μ changes progressively with temperature like the value of Q_{10}.[6, 49] .

(iv) *That the logarithm of the velocity is porportional to the logarithm of the temperature.*

$$v = \frac{t^k}{a}$$

where v = velocity, t = temperature, k and a = constants.

In many cases where the value of Q_{10} or μ appears to change with temperature, and breaks occur in curves plotted according to (ii) and (iii) above, a straight line is obtained when the results are plotted in accordance with this empirical equation (as in the development of *Dytiscus* [8]; and the rate of metabolism in the different stages of *B. mori* [5]). When the exponent k is equal to 1, this formula becomes identical with (i) above ; it will therefore embrace cases where there is a linear relation between temperature and velocity.[6]

(v) *That the destructive action of temperature at the upper limit of the temperature range also follows the van't Hoff rule.* All the preceding equations apply only to the ascending part of the velocity curve ; they take no account of the fall in the curve near the lethal temperature. According to Duclaux's theory of the effect of temperature on enzyme action, both the accelerating and destructive effects of rising temperature follow the van't Hoff rule, and the ordinary velocity-temperature curve represents the sum of these two effects. This same idea has been applied to the effects of temperature on insect metabolism [71] and rate of development.[39] The curve relating time of development with temperature (Fig. 287, *A*, *a*) would therefore be regarded as a catenary curve, representing the sum of two exponential curves $y = ma^x$ and $y = ma^{-x}$, the complete equation being

$$y = \frac{m}{2}(a^x + a^{-x})$$

where y = time required for development, m = the time at the optimum, x = excess of temperature in °C. above the optimum and a = a constant. The graph of the reciprocal gives the relation between velocity of development and temperature ; and by various modifications in the equation, it is possible (after the experimental values have been obtained over the entire range) to obtain a fairly good fit between the curve and the experimental points.[39] Good agreement, for example, can be obtained in the case of egg development in *Sitona* [2]; fairly good agreement with the rate of crawling of *Lymantria* larvae.[14]

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Chapter XV

Reproductive System

REPRODUCTION IN most insects is bisexual; the egg cell liberated by the female will develop only after fusion with the spermatozoal cell set free by the male. The physiology of reproduction deals with the arrangements for the separation and ripening of these male and female gametes, and with the mechanisms by which they are brought together. The reproductive system consists of paired sexual glands, the ovaries of the female and testes of the male, paired gonoducts of mesodermal origin into which the sexual products are discharged, and a median duct lined with cuticle, derived by invagination from the ventral body wall, forming the vagina in the female and the ejaculatory duct in the male.

FEMALE REPRODUCTIVE SYSTEM

The ovary—Each ovary is made up of a series of egg tubes or ovarioles, varying in number from one in the viviparous Diptera such as *Glossina*, and in some Aphids, to more than 2,000 in certain termites. The ovarioles consist of a chain of developing ova (Fig. 290). Each is composed of a layer of epithelium resting on a basement membrane, the whole enclosed in a connective tissue coat. They are divided into the following zones.

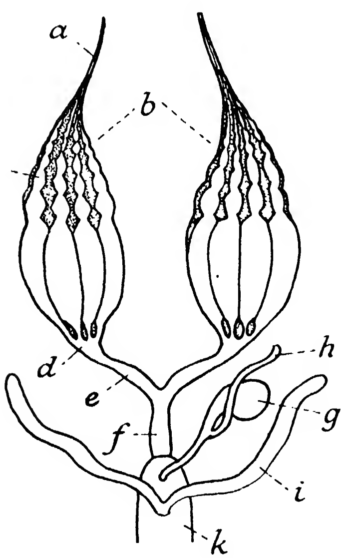

FIG. 290.—Female reproductive system, diagrammatic (*after* SNODGRASS)

a, ovarial ligament; *b*, ovary; *c*, ovariole; *d*, calyx; *e*, lateral oviduct; *f*, common oviduct; *g*, spermatheca; *h*, spermathecal gland; *i*, accessory duct; *k*, vagina.

(i) *Terminal filament*, a thread-like continuation of the connective tissue layer, which often attaches all the ovarioles to the body wall.

(ii) *Germarium*, consisting of densely packed cells from among which the primordial germ cells or oögonia become differentiated into oöcytes and nutritive or nurse cells.

(iii) *Vitellarium*, composing the greater part, which consists of a series of oöcytes, each enclosed in an epithelial sac or follicle, becoming progressively larger towards the lower end.

(iv) *Ovariole stalk*, a thin-walled tube leading to the oviduct. While the leading oöcyte is ripening it is separated from the lumen of this tube by a solid plug of epithelium. This plug is broken down during ovulation (p. 380).

Two types of ovarioles are recognized: (*a*) *Panoistic type*, in which the nutritive cells are wanting, and the yolk of the egg is formed solely by the epithelium of the egg follicles (Fig. 291, A). This is probably the primitive type, but it has been secondarily acquired by many forms such as Ephemeroptera, and many Orthoptera. (*b*) *Meroistic type*, in which nutritive or nurse cells as well as germ cells arise from the primitive sex cells and contribute to the nourishment of the oöcytes in the early stages of their development. This type is divided into two sub-groups: the *polytrophic group*, in which each oöcyte has a number of nurse cells enclosed with it in its follicle, as in Dermaptera, Lepidoptera, Diptera, Hymenoptera, Coleoptera-Polyphaga (Fig. 291, B); and the *acrotrophic* or *telotrophic* group, in which the nurse cells are confined to the apex of each ovariole and are connected to the developing oöcytes in the early stages of their development by means of long nutritive cords, as in Hemiptera, Coleoptera-Adephaga, Aphaniptera.

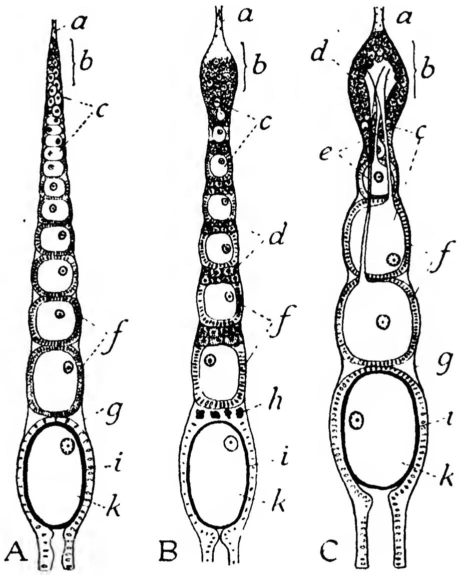

FIG. 291.—The three chief types of ovaries, diagrammatic (*after* WEBER)

A, panoistic type; *B*, polytrophic type; *C*, telotrophic type. *a*, terminal filament; *b*, germarium; *c*, oöcytes; *d*, nurse cells; *e*, nutritive cords; *f*, follicular cells; *g*, peritoneal coat; *h*, degenerated nurse cells; *i*, chorion; *k*, egg.

Genital ducts and accessory structures—The paired oviducts derived from the mesoderm, sometimes, as in Lepidoptera, distended to form pouches for storing eggs, unite with the common oviduct, which is lined with cuticle continuous with the body surface. The common oviduct is generally termed the vagina; in some Diptera its anterior part, which has well-developed muscular walls, is called the uterus. Associated with the vagina are the following:

(i) The *receptacular duct* leading to the *spermatheca* or *receptaculum seminis*. This is usually a fine duct opening on to the dorsum of the vagina. The spermatheca is a pouch lined with cuticle in which the spermatozoa received at copulation are stored. It may contain glands. It is usually single; but there are two in *Blaps* (Col.), *Phlebotomus* and *Dacus* (Dipt.), three in *Culex*, Tabanidae and most higher flies.

(ii) The *bursa copulatrix* into which the sperm are discharged before entering the spermatheca. It is often absent. When present it generally forms a diverticulum from the vagina; and then the bursa or its duct usually receives the receptacular duct. In the higher Lepidoptera, the external opening of the bursa, used for copulation, is quite separate from the vaginal opening through which the eggs are laid (Fig. 292).

(iii) *The accessory glands*, of which there are usually one or two pairs, open into the distal portion of the vagina. They are often termed ' colleterial glands ', since they commonly produce an adhesive cement. But they have many other functions (p. 381).

All these structures show great diversity in the details of their arrangements among different insects.[161]

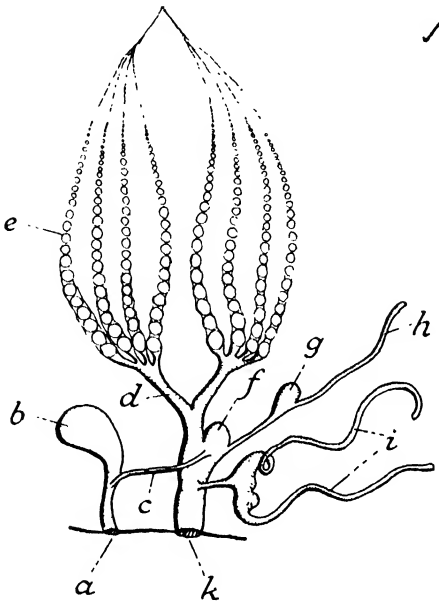

FIG. 292.—Female reproductive system in Lepidoptera; schematic (*after* EIDMANN)

a, ostium of bursa (copulatory opening); b, bursa copulatrix; c, seminal duct; d, paired oviduct; e, ovary; f, vestibulum; g, receptaculum seminis; h, receptacular gland; i, accessory glands; k, ovipore.

Oögenesis—The oögonia in the end chamber or germarium become differentiated into oöcytes and nurse cells. As the oöcytes move backwards they become surrounded by the 'follicle cells' of somatic origin, which compose the hind part of the germarium, and each oöcyte in its follicle forms an egg chamber of the vitellarium. At first the follicle is several cells thick; as the oöcyte grows the cells finally arrange themselves in a single layer.

When nurse cells are present there may be a single one to each egg as in Dermaptera, 5 as in Lepidoptera, or up to 48 as in the queen bee.[26] They obtain nourishment from the blood and pass it on to the oöcyte. In Lepidoptera the streaming of secretion from the nurse cells into the oöcyte is clearly visible in histological sections (Fig. 293).[137] In Hemiptera with their telotrophic ovaries, the nutrient cords extend into the oöcytes from a cavity in the germarium surrounded by nurse cells; these cords are probably formed chiefly by the nurse cells, though perhaps in part by the oöcytes themselves.[27] In the polytrophic ovaries the nurse cells ultimately break down completely and are absorbed into the egg. In the telotrophic type the nutrient cords are severed as soon as the oöcyte has grown to a certain size.

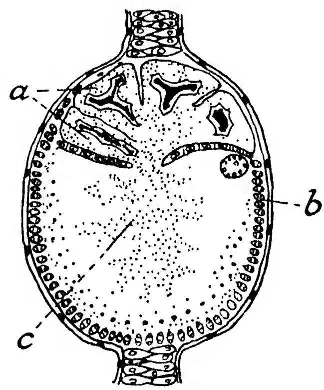

FIG. 293.—Longitudinal section of oöcyte of *Deilephila* at the height of the secretory activity of the nurse cells (*after* SCHNEIDER)

a, nurse cells; b, follicular cells; c, secretion from nurse cells streaming into the egg plasma.

In the later stages of their growth the eggs are nourished by secretion from the follicular cells. At this time the follicular cells become greatly enlarged, and the ratio of plasma to nucleus increases. The nuclei may become constricted in the middle, as in Phasmids [21] and *Pediculus*,[37] or they may divide amitotically into two, in many insects. But this nuclear fission never leads to division of the cells [80]; it is perhaps simply a mechanism for increasing the nuclear and nucleolar surface during the phase of intense secretory activity.[104] Such amitosis is not uncommon among cells which are approaching the end of their functional life.[50]

Yolk formation—As soon as the oöcyte begins to enlarge beyond its sister

nurse cells, granules and vacuoles of reserve material make their appearance in the cytoplasm. These yolk spheres doubtless have a different composition in different insects; but they consist in the main of proteins, fats and carbohydrates. Droplets or granules of these materials can never be demonstrated in the nurse cells or in the follicular cells; they appear to be formed within the oöcyte from the secretions with which it is provided.[130] The fatty yolk droplets arise by the deposition of fat within the Golgi vacuoles of the oöcytes, in *Periplaneta*,[49, 106] *Luciola* (Col.),[105] Tenthredinidae.[49] The protein spheres arise from basophil granules, which appear in the neighbourhood of the nucleus, and subsequently migrate to the periphery and enlarge. These granules are sometimes referred to as 'chromidia'; in Anoplura they are said to arise exclusively from the chromosomal chromatin (though they fail to give the

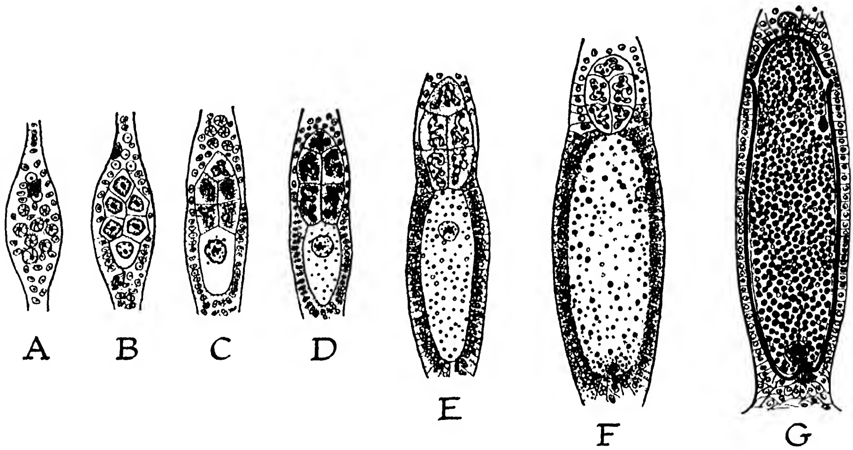

FIG. 294.—Oögenesis in polytrophic ovary of *Anoplura*, semischematic (*after* RIES)

A, stage showing six undifferentiated oögonia. *B*, the six cells differentiated into five nurse cells and one oöcyte; follicular epithelium forming. *C*, the enlarged nucleus of oöcyte giving off chromidia to the plasma; nurse cells beginning to function; next group of oögonia visible above. *D*, nuclei of follicular cells assume dumb-bell form; nurse cells at height of activity; plasma with numerous chromidia; *E*, follicular epithelium active; nurse cells begin to regress; chromidia formation ceases; fat droplets appear in plasma. *F*, follicular epithelium at height of activity; symbionts invade lower pole of egg; nucleus of oöcyte dissolved, chromosomes free in plasma at periphery of egg; yolk formation begins. *G*, ripe egg with reserve materials complete; follicle cells have laid down chorion; mass of symbionts at lower pole; nurse cells degenerated; spindle of first maturation division formed.

Feulgen reaction for nuclein) and to be shed off solely from the nucleus of the oöcyte itself (Fig. 294, C).[130] In *Periplaneta* [61, 106] they arise by the extrusion of buds from the nucleolus of the oöcyte. They then migrate to the cytoplasm, and thence to the periphery of the egg. Here they grow and break up into small chromatic bodies, which enlarge to produce the albuminous yolk spheres.[49, 61, 106] A similar process occurs in Tenthredinidae [49]; but here the so-called 'accessory nuclei' are extruded from the nuclei of the nurse cells and follicular cells as well as by budding from the nucleolus of the oöcyte itself.[117] In *Luciola* the nucleolar budding continues throughout oögenesis.[105]

In Anoplura the chromidia are formed very early; the fat droplets appear next. The former grow to produce the yolk spheres and the latter appear in abundance during the time when the follicular cells reach the height of their

activity and the nurse cells are degenerating. Glycogen is diffused throughout the egg substance; its appearance coincides with the completion of yolk formation when the follicular cells are beginning to secrete the egg-shell (Fig. 294).[130]

Egg membranes—When the yolk is fully formed, the vitelline membrane (p. 1) arises by the condensation of its outermost layer; and the chorion is secreted by the follicular epithelium (Fig. 295). The chorion often shows in its surface sculpturing the impress of the follicular cells; it frequently has outgrowths of characteristic form, a detachable cap at the anterior end, or other more or less elaborate structures which indicate a high degree of co-ordination among the epithelial cells by which they are laid down.[10] For example, in some species of Phasmids, after one chorion has been laid down, the follicle is thrown into longitudinal folds; it then smoothes itself out and lays down a second sheath, attached to the first only at the operculum which marks the position of the micropyle.[21]

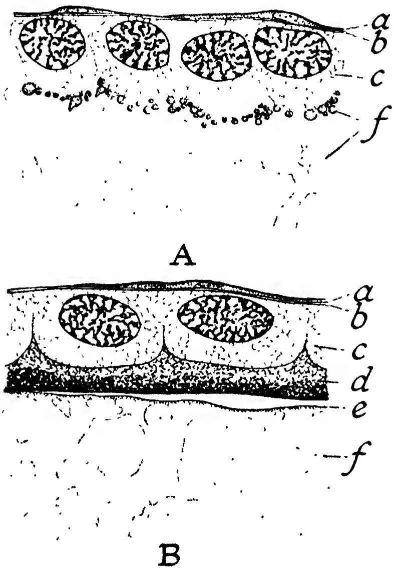

FIG. 295.—Follicular epithelium of *Melanoplus differentialis* (Orthoptera)

A, during the deposition of yolk; B, during the secretion of the chorion (*cf.* Fig. 1). (*After* SLIFER.) a, accessory membrane; b, outer limiting membrane; c, follicular epithelium; d, chorion; e, vitelline membrane; f, yolk.

Micropyle—At one point the chorion is either very thin and adherent to the vitelline membrane, so that the spermatozoa may enter after the shell is formed, or there may be actual perforations in the chorion. In Muscidae there is an entomicropyle penetrating the vitelline membrane, an ectomicropyle or perforation of the chorion, with an area of modified sculpturing around, and a soft 'mucous' plug projecting to the exterior.[113] The number of openings varies even within a single species. In *Locusta* the apparatus consists of a ring of 35–43 funnel-shaped cuticular canals which taper inwards and open to the interior of the egg by fine apertures in the vitelline membrane.[133] In Locustids, the canaliculi run obliquely through the chorion; they vary in number from 1–23 in different species.[20]

Ovulation; 'corpus luteum'—When the leading oöcyte is ripe, it ruptures the epithelial plug which closes the vitellarium below, and passes into the oviduct. This epithelial plug does not regenerate in its original form.[50] The empty follicle collapses, the cells degenerate, undergo autolysis and gradually disappear (Fig. 296). In the early stages of this process the degenerating follicle is a conspicuous object and is known as the 'corpus luteum'; but it shows no progressive development after ovulation like the corresponding organ in mammals. In termites, the cells close to the autolysing follicle may contain deposits of a bright yellow pigment, but this has no relation with the lipochrome lutein of vertebrates.[3] As the eggs above enlarge and extend downwards this body shrinks, and by the time the next egg is ripe it has almost

disappeared. When this egg is ovulated it ruptures its follicle and passes through the remains of the preceding corpus luteum.[50]

The mechanism of ovulation is not understood. In many insects, for example Lepidoptera, the eggs are set free in rapid succession as they are formed, independently of any external stimulus. In *Glossina*, ovulation seems to take place only after impregnation, the central nervous system perhaps controlling the process; thus, normally, one egg is produced at a time; but virgin females 4 or 5 weeks old may contain three or four fully developed eggs in the ovaries; these are ovulated within three days after fecundation.[98]

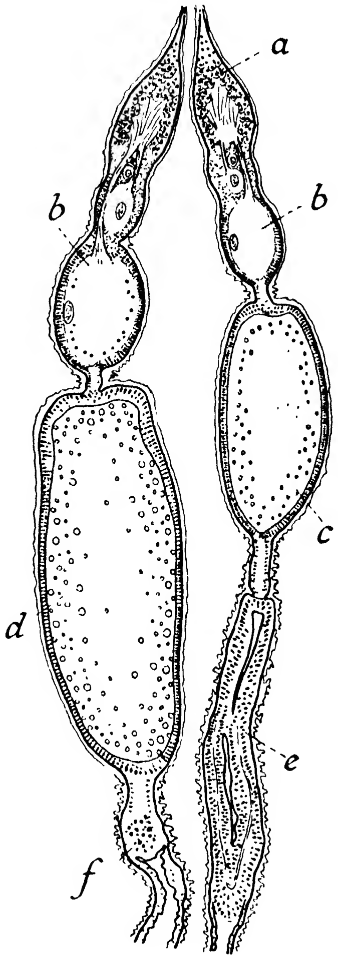

FIG. 296.—Egg development in *Rhodnius* (*after* WIGGLESWORTH)

a, nurse cells; *b*, oöcytes nourished by nutritive cords from nurse cells; *c*, oöcyte nourished solely by follicular epithelium; *d*, egg almost fully developed; *e*, empty follicle, or corpus luteum; *f*, last remains of corpus luteum.

Oviposition—The eggs are carried down the oviducts by waves of peristalsis, and deposited singly or in masses according to the habits of the species. They are generally coated with secretion from the accessory glands; as was suggested by Malpighi, this causes them to adhere to the surface on which they are laid, as in Lepidoptera,[33, 110] Hemiptera,[42] &c. Sometimes these glands provide a gelatinous sheath in which the eggs are embedded, as in the aquatic Trichoptera and Chironomidae. They may be transformed into silk glands by which an elaborate 'egg cocoon' is formed, as in Hydrophilidae. In the cockroach, the eggs are retained in the lower end of the vagina, and cemented together by a thick fluid containing calcium oxalate secreted by the accessory glands; this hardens in the air to form the shell of the egg capsule or oötheca.[52] Gummy material from the accessory glands forms the oötheca of Mantids; it is worked into an elaborate structure, the outermost layers being mixed with air to form a froth which hardens to produce a vacuolated sheath.[170] In Acridiids the oötheca is contributed by glands in the mesodermal part of the oviducts; but the buried egg mass is covered with a foamy secretion from the accessory glands, which hardens to form a plug. In Hymenoptera the accessory glands are modified to form (i) the poison gland which sometimes serves, in Pompilids and Sphecids, to paralyse the prey on which the larva is to feed, and (ii) the so-called 'alkaline' or Dufour's gland which serves perhaps to lubricate the ovipositor or sting, perhaps as a 'colleterial gland' (p. 331).

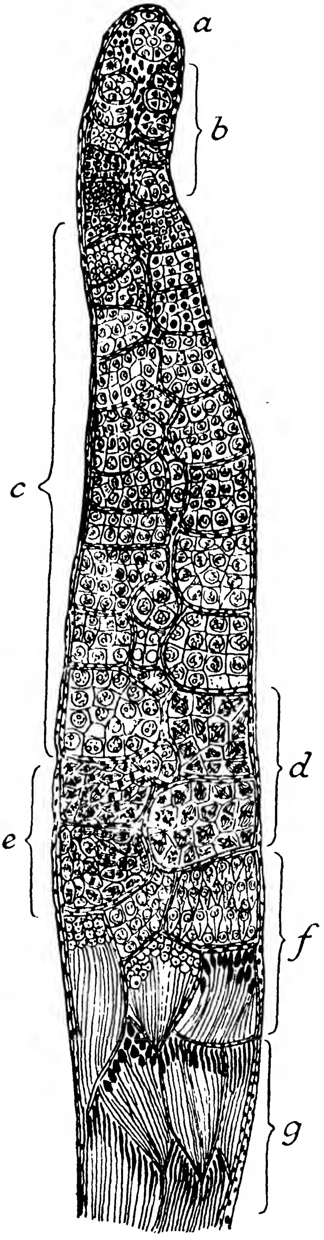

FIG. 297.—Longitudinal section of testis follicle of an Acridiid, semischematic (*from* WEBER *after* DEPDOLLA)

a, apical cells surrounded by spermatogonia; *b*, zone of spermatogonia; *c*, zone of spermatocytes; *d*, cysts with mitoses of first maturation division; *e*, cysts with mitoses of second maturation division; *f*, zone of spermatids; *g*, zone of spermatozoa.

MALE REPRODUCTIVE SYSTEM

Testis and spermatogenesis—The testis is made up of a series of tubular follicles varying greatly in number and arrangement in different insects.[161] Each follicle consists at first of a layer of epithelium resting on a basement membrane. Later, the entire group of follicles is enclosed in a connective tissue sheath, often pigmented, which determines the outward form of the organ. In Lepidoptera the two testes are bound together in a single capsule.

Each follicle contains a succession of zones in which the sex cells are in different stages of development (Fig. 297). These are (i) the *germarium* or *zone of spermatogonia*, consisting of densely packed primordial germ cells or spermatogonia, lying among somatic mesodermal cells. At the upper extremity of the follicles in Lepidoptera and some other insects large 'apical cells' are present which serve as nurse cells in the early stages of sperm development.

(ii) *Zone of spermatocytes.* As each primitive spermatogonium moves backwards, it becomes covered with a mantle of somatic cells; meanwhile it divides repeatedly and distends this sheath to form a cyst. At first the cysts are rounded, but as they increase in size they become polyhedral from mutual pressure. Each contains the 'spermatocytes', from 64 to 256 in number, derived from a single spermatogonium.

(iii) *Zone of maturation and reduction.* The spermatocytes now divide, first into 2 'prespermatids', and then into 4 'spermatids'. In most insects the first of these is the reduction division at which the chromosome number is halved.

(iv) *Zone of transformation*, in which the compressed and rounded spermatids, still enclosed in their cysts, are converted into flagellated spermatozoa.

The cytology of this process of spermatogenesis has been studied in great detail in many insects.[27] The mature sperm break through their cyst wall to enter the genital ducts by means of the periodic writhing movements of their flagella. At this stage they are often still held together in bundles (spermatodesms) of various forms, their heads being inserted into a cap or rod

of gelatinous material. In the grasshopper *Chortophaga* this hyaline cap remains intact until the bundles reach the seminal receptacle of the female ; here it is dissolved, probably by enzyme action, and the spermatozoa set free.[116] In *Bombyx mori* the spermatozoa in the testis become active, perhaps under the influence of some chemical substance from the lower part of the tract, and wriggle in bundles through the basilar membrane which separates the testis lobules from the efferent duct, the bundles being broken down when they come in contact with the secretion of the accessory glands.[111] In most insects, mature spermatozoa have already left the testis at the time of emergence from the pupa.

Genital ducts and accessory structures (Fig. 298)—These consist essentially of fine paired ducts, ' vasa deferentia ', which are either dilated at one point (' vesicula seminalis ') or provided with a tightly coiled middle section (the ' accessory testis ' of many Coleoptera) so as to furnish a reservoir for the mature sperm. The vasa deferentia unite to form a common duct continuous with the median ' ejaculatory duct ' of ectodermal origin. This is lined with cuticle and provided with a powerful muscular coat made up of outer circular and inner longitudinal fibres. Its terminal part is often enclosed in an evagination of the body wall to form the intromittent penis or ' aedeagus '. ' Accessory glands ' of varied number and form discharge into these ducts ; in addition, in some insects, parts of the ducts themselves may be glandular in function.

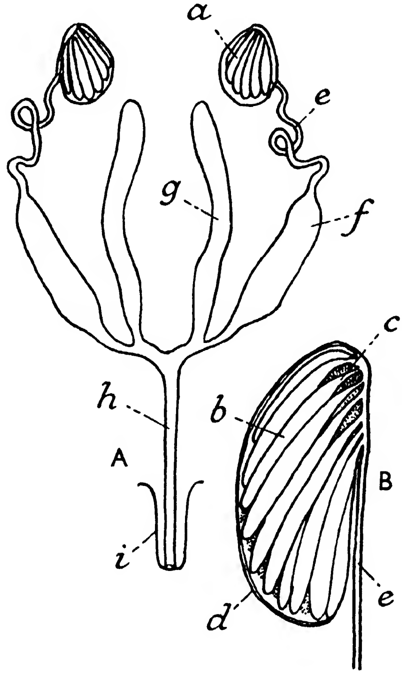

Fig. 298

A, male reproductive system, diagrammatic. *B*, detail of testis. (*After* Snodgrass.) *a*, testis ; *b*, follicles ; *c*, vas efferens ; *d*, peritoneal sheath ; *e*, vas deferens ; *f*, vesicula seminalis ; *g*, accessory glands ; *h*, ejaculatory duct ; *i*, penis.

MATING, IMPREGNATION, AND FERTILIZATION

Mating—The sexual behaviour of insects is apparently not influenced by the gonads. Females of Aleurodidae copulate soon after emergence, long before the ovaries are fully developed.[160] Castrated males of *Lymantria* will copulate normally, although no spermatozoa are passed [112] ; and males and females of *Gryllus* will mate in the usual way after castration.[125] In male *Dytiscus*, sexual activity seems to be due to a nervous impulse resulting from distension of the accessory glands.[11]

Stimuli of many kinds provide the immediate cause of mating. The females may be attracted to the males by their dancing in swarms, as in various Diptera-Nematocera, Trichoptera, Plecoptera and Ephemeroptera ; or by sounds, such as the chirping of crickets and grasshoppers.[125] Males may be attracted from a distance by the luminous organs of the female, as in Lampyrid beetles.

Among butterflies, the colour of the female may attract the male when he is in the right physiological state. The female may be excited by a complex ceremonial love play.[161] But the most important stimuli are probably scents.[128]

Scent organs in mating—Scent organs in the female may attract the male from a distance. Such organs are particularly common among Lepidoptera. Here they occur in the neighbourhood of the sexual opening and take the form either of tufts of modified scales, or hairs with gland cells at their base, or a simple fold in the body wall consisting of a glandular epithelium covered by thin cuticle devoid of pores (Fig. 299).[40] Such organs are said to be best developed among those Lepidoptera, such as Lasiocampidae and Bombycidae, in which the eggs of the female are ripe for laying at the time of emergence from the pupa.[33] Males of *Arctias selene* liberated at a distance of 11 km. were able to return to the females.[97] Males of *Callosamia promethea* are readily attracted to the isolated abdomen of the female, or to places where a recently emerged female has rested; they pay no attention to females in a glass container or deprived of the abdomen, and they are not repelled by any abnormalities produced experimentally in the colour or pattern of the wings.[95] Males of *Bombyx mori*,[75] and of *Ephestia* and *Plodia*[30] show the same behaviour. Males of *Orgyia* will endeavour to copulate with pieces of blotting paper on which the droplets of secretion from the everted gland of the female have been imbibed[40]; and males of *Habrobracon* (Hym.) are attracted not only to females, but to other males if these have recently copulated.[103]

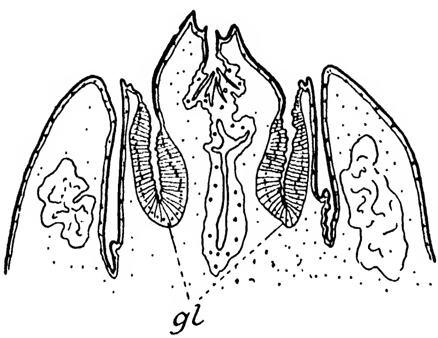

Fig. 299.—Horizontal section through tip of abdomen of *Plodia* female, showing intersegmental scent gland (*gl*) (*after* Barth)

Females of Lepidoptera awaiting fecundation take up a characteristic 'calling' position, the stimulus to which seems to be provided by a shortage of sperm in the receptaculum.[110] Only during the assumption of this attitude are the scent-producing membranes exposed.[30] Presumably the scent produced by the female in these cases is characteristic of the species; but in confinement the males of the various species of *Ephestia* and *Plodia* are attracted to 'calling' females of other species, and make attempts to copulate with them[7, 30]; and the same is true of the related genera *Galleria* and *Achroea*.[7] Males of the Trypetid fly *Dacus*, are attracted to specific essential oils: *D. zonatus* to methyl-eugenol, *D. diversus* to isoeugenol, *D. ferrugineus* to both, but especially the latter, while eugenol itself is not attractive to any.[65] Perhaps this is a response to an odour approximating to that of the female.

The males of many Lepidoptera, also, have scent producing organs: tufts of glandular scales or hairs on the abdomen, as in Phycitidae[30]; or on the legs[68]; or as 'androconia' scattered or concentrated in patches on the wings.[40, 68] In some cases the pencil of hairs used for dispersing the scent is separated from the glandular area by which it is secreted; as in some Danaine butterflies,[34] or in the abdomen of certain Noctuids and Sphingids.[149] These male scents are generally regarded as having an aphrodisiac function,

exciting the female to copulation; in the case of *Ephestia* there is some slight experimental evidence for this belief.[30]

Feeding habits during mating—There are many peculiar habits associated with mating, the physiological significance of which is uncertain. Female *Panorpa* eat secretory globules produced by the salivary glands of the male; the males of certain flies regurgitate a droplet of fluid for the female; the males of Empidae provide the female with food in the form of prey, enclosed by some species in a silk wrapping, while in others the wrapping alone is provided; the females of the tree cricket *Oecanthus* feed on a secretion from the metanotal glands of the male. These habits are sometimes thought to be related to the need of the egg-laying female for a protein diet [128]; in some cases they seem to serve the purpose of protecting the spermatophore (p. 387), or the male himself, from being devoured. Mantid females are particularly liable to eat the male; and it is interesting to recall (p. 103) that removal of the head during such an attack actually stimulates the male to copulation, since the copulatory centre in the last abdominal ganglion is free from the inhibitory influence of the suboesophageal ganglion. Normal pairing, with the transfer of a spermatophore, may take place even when both insects are decapitated.[132]

Transfer of sperm—The sexes often remain connected for several hours, held together in some cases by highly complex structures, while the transfer of the sperm takes place. In a few species, such as the butterfly *Parnassius*, the hypertrophied accessory glands of the male produce a secretion which hardens and cements them together during this period.

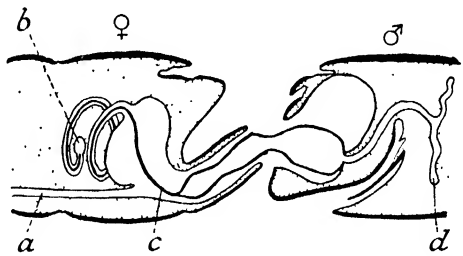

Fig. 300.—Copulation in *Lygaeus equestris*, showing extension of the penis of the male into the receptaculum seminis of the female; schematic (*after* Ludwig)

a, oviduct; *b*, receptaculum seminis; *c*, penis; *d*, vesicle containing fluid which is driven into the penis in order to extend and uncoil it.

In some insects, such as the bug *Lygaeus*, the penis after entering the vagina penetrates the duct of the receptaculum, so that the spermatozoa, mixed with the secretion of the accessory glands to form a viscous fluid mass, are ejaculated directly into the spermatheca (Fig. 300) [88]; more often, as in *Drosophila*,[109] they are discharged into the vagina; or, as in Lepidoptera, Orthoptera, and many Coleoptera, &c., into the bursa copulatrix. Collembola smear the sperm on the mouth parts and introduce them by this means into the genital opening of the female. Males of Odonata eject them into a special apparatus below the base of their own abdomen; the ligula of this apparatus is then inserted into the vagina of the female. In *Cimex* the spermatozoa are discharged into a pouch on the lower surface of the female abdomen; they reach the genital tract by wandering through the general body cavity (p. 388).

Spermatophores—In Lepidoptera,[110] many Orthoptera (Locustids and Gryllids,[12, 43] Acridiids, Mantids, and Phasmids [23]), Neuroptera,[32] Coleoptera, &c. the sperm are not conveyed to the female in a free fluid, but are enclosed in a membranous sac or 'spermatophore', formed by the secretion of the male accessory glands. The spermatophore is never transferred to the receptaculum; it merely serves as a provisional sheath which is deposited in the bursa or vagina.

In *Thermobia* and other Thysanura the spermatophore is simply dropped by the male in the course of a ' love dance ' ; it is then picked up by the female and inserted in the vagina.[150]

In Lepidoptera the spermatophore consists of a round sac of cuticular substance with a narrow neck. At the end of the neck are solid transparent horns of the same material, which correspond exactly with the diverticula from the male ejaculatory duct of the species in question. Near the horns is an oval opening in the neck, through which the sperm escape from the sac (Fig. 301, A, B). The formation of this structure in *Plodia* occupies 1–1½ hours, in *Ephestia* 3–4 hours. The male duct has glandular walls throughout much of its length. During the first 15 minutes of mating, in *Plodia*, the secretion from the lower glandular segment flows into the bursal cavity of the female and hardens into a gelatinous mass. The secretion from the middle segment flows into the ejaculatory duct and its diverticula, where it is moulded to form the

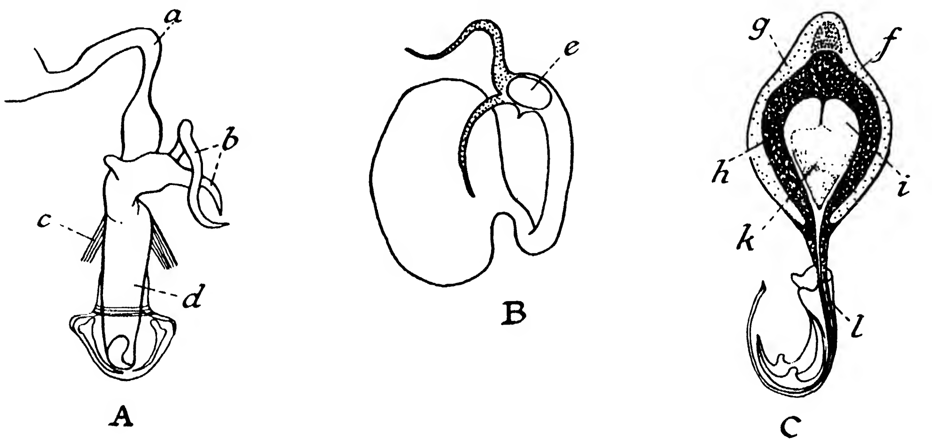

FIG. 301

A, lower part of male reproductive tract in *Ephestia kühniella*. *a*, ejaculatory duct ; *b*, diverticula or horns on ejaculatory duct ; *c*, protractor penis muscle ; *d*, aedeagus. *B*, spermatophore of *Ephestia kühniella*, showing horns moulded in the diverticula of the ejaculatory duct. *e*, aperture in neck of spermatophore through which the sperm escapes. (*After* NORRIS.) *C*, diagrammatic longitudinal section of spermatophore of *Liogryllus campestris*. *f*, outer sheath ; *g*, middle layer ; *h*, inner layer ; *i*, substance which presses out the sperm ; *k*, mass of sperm ; *l*, sperm tube. (*After* REGEN.)

horns and neck. The secretion from the uppermost glandular segment is then forced down, pushing the soft core of the previous secretion before it into the bursa. The sac is further distended by the sperm, and by the secretion of the accessory glands, which follow. Finally the neck of the spermatophore contracts and is withdrawn from the ejaculatory duct of the male into the bursal cavity of the female. Here it is always orientated so that the aperture of the neck lies near the entrance of the ductus seminalis.[110] In some Lepidoptera several spermatophores (up to nine) may be deposited in the bursa during copulation.[33]

In the cricket *Oecanthus*, the spermatophore is produced rather differently. The atrium of the male system is first distended with the mass of sperm ; around this the numerous accessory ducts then pour out their secretion, forming a covering like the shell of an egg. The soft mass is driven by peristalsis into the penis cavity, becoming globular ; and finally the terminal filament and the lateral lamellae are formed by the secretion flowing into the penis groove

and there rapidly hardening.[62] Only the neck of the completed sac is introduced into the vagina of the female; it is anchored there by the lateral lamellae, and often secured in position by a secretory plug applied by the male. Castrated males of *Gryllus* will produce and transfer empty spermatophores of this type during copulation.[125]

The spermatophore is emptied of sperm soon after it has been inserted. In *Dytiscus*, in which it is deposited at the entrance of the vagina, they are perhaps squeezed out by pressure from the terminal sternites of the abdomen; emptying in this case is not complete.[11] The spermatophore of Lepidoptera is also thought by some authors to be emptied by the pressure of the bursa.[33, 110] But this cannot be the explanation in Orthoptera, in which the body of the spermatophore remains outside the genital opening. In *Liogryllus* the wall of the capsule is made up of a thin outer, a thicker middle, and a still thicker inner layer; inside this is a gelatinous body which leaves only a relatively small cavity for sperm (Fig. 301, C). During emptying, it is believed that the gelatinous body absorbs water from the middle layer of the capsule and swells to fill the lumen and force out all the spermatozoa.[126] In *Oecanthus* this process is completed in about 15 minutes; during this time the female is feeding on the metathoracic secretion of the male; when that is finished she removes the empty spermatophore and devours it.[62] Perhaps emptying is brought about in the same way in Lepidoptera, the inner layers taking up water from the accessory gland secretion which contains the sperm.[57]

Migration of spermatozoa to the receptaculum—Whether the sperm are discharged into the female directly or by way of a spermatophore, they are

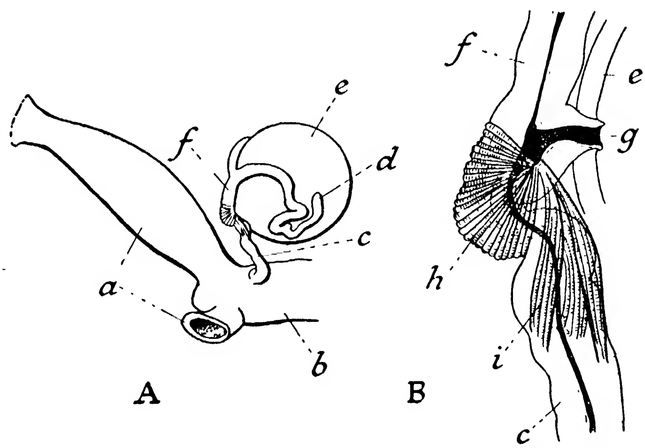

FIG. 302.—Sperm pump of queen bee (*after* BRESSLAU)

A, general anatomy of spermatheca; *B*, detail of entry of spermathecal duct into spermatheca. *a*, oviducts; *b*, vagina; *c*, duct of spermatheca; *d*, spermathecal gland; *e*, spermatheca; *f*, duct of spermathecal glands; *g*, orifice of spermathecal duct into spermatheca; *h*, semicircular muscles of sperm pump; *i*, longitudinal muscles.

next transferred to the receptaculum seminis. The mechanism of this transfer is uncertain. According to some authors it is effected mechanically. The duct of the spermatheca of the bee has a complex muscular apparatus arranged in a semicircular manner round the duct; this has been described as a suction pump which is not only used in fertilization (p. 405) but serves also to transport into the receptaculum the sperm received at copulation (Fig. 302).[2, 15] In

Lepidoptera, the sperm have to pass from the bursa copulatrix, through the ductus seminalis to the common oviduct, and thence by another fine duct to the receptaculum seminis (Fig. 292). This movement, also, has been thought to be brought about by muscular contraction, squeezing by the bursa and aspiration by the receptaculum. The bursa is completely emptied of spermatozoa in 1–3 days.[33, 110]

Other authors regard these mechanical theories as untenable, and consider that migration is the result of active movements by the spermatozoa in response to chemical stimuli.[57] In *Drosophila* the spermatozoa are ejaculated into the uterus. Here they remain motionless for some time; but after 2 or 3 minutes they begin to move, activated perhaps by the secretion of the parovaria or accessory glands; and then they swim actively to the two spermathecae and a ventral diverticulum which serve as seminal receptacles.[109] The unicellular glands in the walls of the receptaculum are thought to be the source of the chemotactic stimulus in *Lygaeus*,[88] the receptacular gland in Coleoptera [131] and Lepidoptera.[57] But at present there is no experimental proof for these ideas.

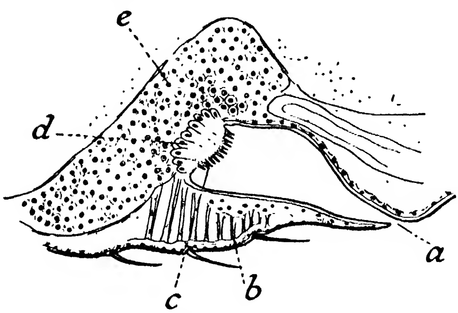

FIG. 303.—Longitudinal section through the fourth abdominal sternite of *Cimex* female showing organ of Ribaga (*after* ABRAHAM)

a, entrance for penis; *b*, muscles; *c*, cuticular processes; *d*, matrix cells of these processes; *e*, cells of organ of Berlese.

Migration occurs by a different route in the bed-bug *Cimex*. The spermatozoa are ejaculated into the organ of Ribaga, a sac on the ventral surface of the abdomen (Fig. 303). They make their way through the floor of this pouch into a mass of underlying cellular tissue, sometimes known as the organ of Berlese. This organ appears to produce some internal secretion which it discharges into the blood when the spermatozoa pass through it.[25] Perhaps it serves to activate the sperm.[1] These reach the body cavity in 2–3 hours and then migrate, perhaps by chemotaxis, to a pair of evaginations ('spermathecae'[25] or 'absorptive organs'[1]) at the upper end of the common oviduct, which they reach in 2–10 hours.

Migration of spermatozoa into the egg—Fertilization may not take place until long after copulation (p. 390). Usually it occurs immediately before the egg is laid. A few spermatozoa leave the receptaculum and enter the micropyle of the egg just as it passes the receptacular duct. Various mechanisms are concerned in controlling this process.

(i) In the first place the egg is so orientated that the micropyle comes to lie exactly opposite the mouth of the duct. This is clearly seen in *Periplaneta*,[29] Lepidoptera (*Plodia* and *Ephestia*),[110] and *Drosophila* (Fig. 304).[109] In Vespids there is a dilatation of the vagina at this point, in Apids an evagination closed by a flap, and in Formicids a definite 'fertilization pouch', all concerned in directing the cephalic pole of the egg, bearing the micropyle, towards the duct of the spermatheca.[2]

(ii) The discharge of sperm may be under muscular control. Many Coleoptera and Hemiptera have a compressor muscle in the spermatheca

(Fig. 305)[57, 159]; in the grasshopper *Chortophaga* the sperm are ejected by contraction of the terminal muscular portion of the seminal duct[116]; in some

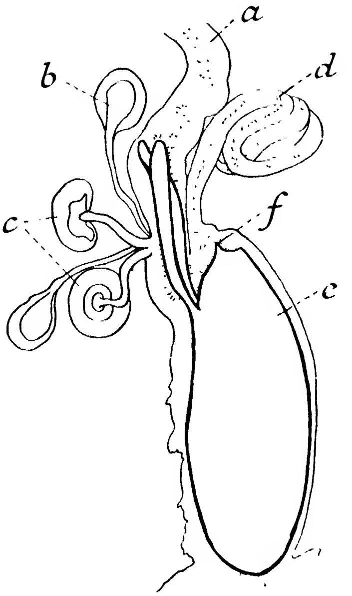

FIG. 304.—Egg of *Drosophila* in vagina at moment of fertilization (*after* NONIDEZ)

a, common oviduct; *b*, accessory gland; *c*, spermathecae; *d*, ventral receptacle; *e*, egg; *f*, micropyle.

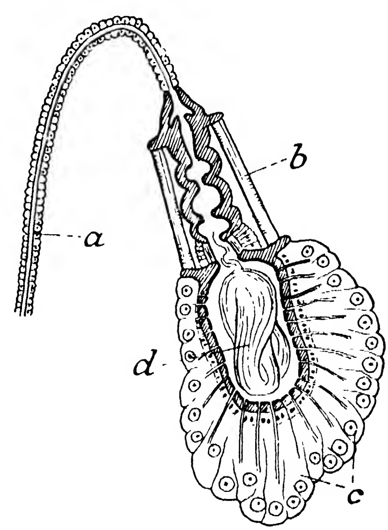

FIG. 305.—Spermatheca of *Graphosoma* (Heteroptera) showing the compressor muscle and the glandular walls (*after* BERLESE)

a, duct of spermatheca; *b*, compressor muscle; *c*, glands with intracellular ducts piercing the cuticular lining of spermatheca; *d*, mass of sperm.

cases perhaps there is a reflex increase in blood pressure when the egg enters the posterior part of the vagina, which may serve to squeeze the spermatheca,[57] or a reflex dilatation of the orifice of the seminal receptacle.[109] In the honey-bee, fertilization is certainly under 'voluntary' control (p. 405). Here the muscle around the duct of the spermatheca serves perhaps to pump or aspirate a few spermatozoa (variously estimated at 10[2] or 100[15]) from the sperm pouch to the vagina, or perhaps to control the escape of sperm in response to increased pressure from above.[57]

(iii) The final entry of sperm into the micropyle is probably a chemotactic response.[2, 159] Eggs of the parasitic Muscids isolated from the uterus show a tangled mass of sperm filaments projecting from the micropyle (Fig. 306).[113] In some cases perhaps this chemical stimulation may explain the discharge of sperm from the seminal receptacle[109]; notably where the receptacle has rigid cuticular walls, as in *Leptocoris*.[116]

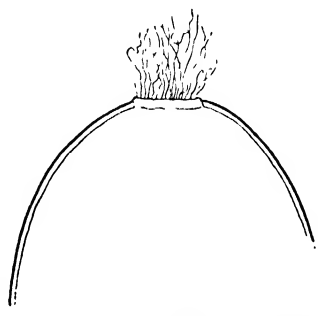

FIG. 306.—Egg of *Fausta radicum* (Tachinidae) extracted from uterus and examined in the fresh state in saline. Micropyle occupied by tangled packets of sperms (*after* PANTEL)

(iv) Spermatozoa of *Periplaneta* always move clockwise in circles and apply

themselves to surfaces. These reactions may assist them in finding the micropyle.[29]

In a few insects the sperm may enter the egg while it is still in the ovary. The spermatozoa of *Cimex*, after reaching the receptacula in the common oviduct, migrate in the substance of the walls of the paired oviducts to the ovarioles, where they appear in great numbers in the follicular epithelium. Fertilization takes place in the ovarioles before the chorion is laid down, probably when the egg is still very small.[1, 25] The eggs of viviparous Chrysomelids, also, are fertilized in the egg follicles by sperm travelling up the oviducts.[127] And in the Polyctenid *Hesperoctenes*, spermatozoa occur free in the body cavity and presumably fertilize the egg in the ovary.[51]

Fertilization—At the time of ovulation, the nucleus of the egg has not yet undergone maturation; but the chromatin has already collected into chromosomes and the first maturation spindle has formed. It remains in this state until the spermatozoon has entered the plasma. This seems to provide the stimulus to maturation. As the spermatozoon approaches the egg nucleus and, losing its tail, becomes converted into the male pronucleus, the two maturation divisions of the female follow rapidly upon one another. One of these is the reduction division which halves the chromosome number. The two divisions result in the formation of the female pronucleus and the polar bodies.

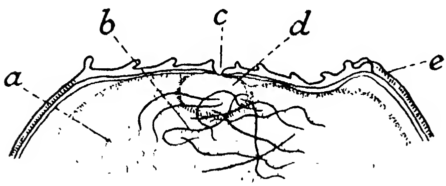

FIG. 307.—Section through upper pole of egg of *Pieris brassicae* (*after* HENKING)
a, yolk; b, spermatozoa; c, micropyle; d, submicropylar region; e, chorion.

Polyspermy seems to be nearly universal among insects; several spermatozoa enter the egg (Fig. 307). Only one of these is normally concerned in fertilization; but where several female nuclei are present, as in the binucleate eggs of Lepidoptera or *Habrobracon* (p. 56) each may be fertilized by a different spermatozoon.[129] In *Drosophila* a few eggs are monospermic; many receive 30 or more spermatozoa. The excess spermatozoa usually degenerate; but in some cases where hundreds have entered the egg they may disorganize the formation of polar bodies, form mitotic figures themselves, or enter into normal cleavages to form multipolar spindles, and upset the egg to such an extent as to prevent further development.[66] It has been suggested that some mosaics and gynandromorphs may possibly arise by the appearance of extra nuclei from spermatozoa which have conjugated.[66]

Fate of excess sperm in the female—The females of many insects mate once only, and the spermatozoa then received fertilize the eggs throughout the oviposition period. In Lepidoptera the sperm remain alive in the receptaculum for several months, nourished and activated perhaps by the secretion from the receptacular gland; even when egg-laying is complete the store of sperm is not exhausted.[33] Females of *Glossina* are impregnated 5–10 days after emergence, and that suffices until their death more than 6 months later.[98] In *Rhodnius* and *Triatoma* a single copulation is sufficient as a rule to ensure fertile ova throughout the entire life of the female; though in some cases the last batches of eggs are sterile in spite of the fact that apparently normal sperm may still be present.[42] And in the honey-bee, the store of sperm received during the nuptial flight lasts for several years.

In a few insects, on the other hand, the sperm which are not immediately used for fertilization are digested and absorbed by the female. This is notably the case in *Cimex*, in which the sperm received at one copulation suffice for egg production for only 4–5 weeks [1]; and at 30° C., if the interval between feeds exceeds 2 weeks, few of the eggs are fertile.[25] Some of the spermatozoa may be absorbed in the cellular organ of Berlese, some by phagocytes in the body cavity, some in the lumen of the common oviduct and in the corpus luteum, but most are digested in the receptacula, which are sometimes described as 'absorptive organs' [1]; here they are completely absorbed in 1–2 weeks. In the bug *Graphosoma lineatum* one segment of the spermatheca apparently serves to take up excess sperm, which are dissolved by the secretion of the male accessory gland, and the products absorbed.[53] According to Berlese's theory of 'hypergamesis', these excess spermatozoa form an important element in the nutrition of the female. In the case of *Cimex*, which copulates repeatedly, it is estimated that the total bulk of the sperm may amount to as much as one-third of that of the eggs laid.[25]

SOME FACTORS CONTROLLING FERTILITY AND FECUNDITY

Many insects with a short imaginal life, such as Ephemeroptera, Plecoptera, many Lepidoptera, copulate once only and lay their eggs in a single batch; others, such as Acridiids, Mantids, Muscids, mosquitos, &c., lay batches of eggs with intervals between, and may copulate repeatedly; others again, such as *Dixippus*, *Tenebrio*, Aleurodids, Aphaniptera, lay single eggs at fairly regular intervals. In this section we shall consider the factors which influence the normal course of egg production in the female, and the factors affecting fertility in the male.

Temperature—The rate of egg production, like other processes of metabolism (p. 366), varies with temperature; it is accelerated up to a point and then falls off rapidly.[160] But the temperature limits between which reproduction can occur are often much narrower than the range of temperature over which the other activities of the same species remain normal. Females of *Locusta migratoria* fail to mature their eggs when the day and night temperatures alternate between 30° and 20° C. [121]; *Pediculus* will not lay eggs below 25° C. [145]; *Anopheles quadrimaculatus* will not lay below 12° C.[96]

The male seems often to be more sensitive than the female to abnormal temperatures. When the Chalcid *Euchalcidia caryobori* was exposed at 16° C. for 10 days, the females still laid the normal number of eggs, but 70 per cent. of the males were sterile.[54] In *Drosophila* kept at 32° C., 50 per cent. of the females were sterile, 96 per cent. of the males; the males were able to copulate but no sperm passed, and the sperm in the male organs lost their motility and subsequently degenerated; if they were returned to the optimum temperature of 24° C. most of the males recovered their fertility.[172] Similarly, when pupating larvae or pupae of *Ephestia kühniella* are kept at temperatures above 27° C. the moths are largely infertile; this is due to an effect on the male; spermatogenesis is retarded and the spermatozoa lose their motility and may disappear.[110, 124]

Nutrition—Fertility in the male is comparatively little affected by nutrition,

though males of *Rhodnius* appear to be less fertile if they are starved in the adult stage,[18] and well nourished females of *Cimex* mated with unfed males produced an average of 45 normal and 12 sterile eggs, as against 153 normal and 42 sterile eggs when mated with males which had been fed.[25] Egg production in the female, on the other hand, may be profoundly influenced by the food supply.

Lepidoptera fall into three not very sharply defined groups according to the state of the ovaries at the time of emergence. (i) In butterflies, and many moths with a long imaginal life, there are very few fully developed eggs; most are matured after emergence. (ii) In most Heterocera there is perhaps a two- or threefold increase in ripe eggs in the imago. (iii) While in a few Bombycids, Lymantriids, &c., all the eggs are fully ripe and no more are developed.[33] We have seen that adult Lepidoptera are unable to assimilate proteins (p. 285); all the protein reserves needed for the eggs must therefore be carried over from the larva; consequently, the fat body of the young imago is massive in group (i), small in group (ii), and already quite used up in group (iii).[33] They can, however, assimilate sugar: if *Agrotis segetum* is fed with a 20–40 per cent. solution of glucose, all the eggs are fertile; if given 5 per cent. glucose forty to fifty per cent. are infertile; and if starved, the number of eggs laid is much reduced, and the embryos all die before hatching. On the other hand, starvation in females of *Loxostege sticticalis* causes less reduction, and in *Pyrausta nubilalis* it has a negligible effect.[82] Similarly, *Ephestia* species do not require sugar; although this increases their longevity it has no effect on their fecundity. But they require water. Fecundity in *E. cautella* and *E. elutella* (at 18–27° C. with moderate humidity) is about halved if they receive no drinking water; it is reduced by less than 20 per cent. in *E. kühniella*. This difference is related with the state of the ovaries at emergence; for *E. kühniella* has already a number of ripe eggs, while *E. cautella* has none.[110] The influence of water and of carbohydrate is seen also in the beetle *Bruchus quadrimaculatus*, in which access to water increases the number of eggs by about 30 per cent., access to sugar and water by about 50 per cent.[84]

In most insects, proteins are essential for egg production. Pteromalid parasites of the weevil *Phytonomus*, when their ovarial follicles reach a certain stage of development, change from a carbohydrate diet to a protein diet consisting of the body fluids of the host species.[38] The house-fly *Musca* requires both sugar and protein as well as water; it lays no eggs if given protein or sugar alone.[45, 135] *Lucilia* females require at least one meal of protein before eggs are laid, whereas this is not necessary for the fertility of the males.[36, 89] In most blood-sucking species, *Stomoxys*,[45] *Haematopota*,[19] *Rhodnius*,[18] *Cimex*,[76] most mosquitos,[171] &c., the number of eggs is determined by the quantity of food. In *Culex pipiens* there is a striking difference between the effects of human and avian blood: only half as many eggs are developed after feeding on man[136]; an average of 121 eggs from 3·0 mg. of human blood, 255 eggs from 3·1 mg. of canary blood.[171] The determining factor in this case is not known.

Among termites, the sterility of the worker and soldier castes, and of the occasional males and females whose gut contains wood particles and protozoa, is attributed to nutrition ('alimentary castration').[165] They do not receive the saliva which seems necessary for the activity of the gonads—perhaps through providing protein, or because it is a source of some specific 'vitamin'.[48] The reverse effect, an activation of the ovaries of the workers, is seen among social

Hymenoptera deprived of their queen. In *Vespa* it is sufficient to suppress the queen for half the workers to become fertile and lay eggs. Having no brood to feed, they reabsorb the food and secretions they would have given to the young ; it is perhaps their occupation as nurses which determines their normal sterility (' nutricial castration ').[93]

Larval nutrition—Since many of the reserves which go to form the eggs are laid down in the larval stage, egg production in the adult may be greatly influenced by the nutrition of the larva. Underfeeding in larvae of *Drosophila*,[4] or *Tineola*,[153] reduces the number of eggs laid. The fecundity of *Ephestia* females is much reduced by feeding the larvae on white flour instead of whole meal, though the average viability of the eggs laid is not affected.[110] In certain races of *Culex pipiens*, the unfed females will develop eggs if their larvae receive a diet rich in proteins, but not after a predominantly carbohydrate diet [58, 164] ; and it is noteworthy that those mosquitos with vestigial mouth parts (*Chaoborus*, *Mochlonyx*) or those which feed only on nectar (*Megarhinus*) are predaceous in the larval stage.[58]

Impregnation—Insects differ in the extent to which impregnation affects egg production. In the louse *Haematopinus*, it seems to have no effect ; egg laying begins three days after the final moult with or without the presence of the male.[39] But that is exceptional. In *Cimex* no eggs are developed until after impregnation [25] ; in *Drosophila*, mating causes an immediate and considerable acceleration in egg production [85]; unmated females of Acridiids produce notably less eggs than mated females,[77] and the same applies to *Musca*.[45]

In many cases the effect of impregnation seems to be on oviposition rather than on egg development. Unmated females of Lepidoptera develop the same number of ripe eggs as mated females, but they lay only about one-third of their total store.[33] Females of *Ephestia* lay only a very few of their eggs when mated with males rendered sterile by high temperatures, most of the eggs being retained in the ovaries.[110] (We have seen that in *Glossina*, impregnation seems to provide the stimulus to ovulation (p. 381).) Lack of copulation reduces the number of eggs laid in *Tineola* [153] ; and in *Lucilia*, copulation appears to provide some essential stimulus for oviposition.[89] What the nature of this stimulus may be is uncertain. In *Lymantria*, oviposition is incomplete after copulation with a castrated male, and yet the bursa is equally distended with the sterile spermatophore as it is after a normal copulation. Distension of the bursa, or chemical stimulation from the secretion of the accessory glands, cannot therefore be responsible. Perhaps the moving sperms themselves provide a tactile stimulus acting through the nervous system.[78]

Internal secretions—It is uncertain to what extent the reproductive activity of insects is controlled by hormones. In *Rhodnius*, ripening of the eggs begins a few days after moulting ; and at this same time the cells of the corpus allatum show signs of renewed secretory activity. If the head, including the corpus allatum, is removed, ripening does not take place ; but if the brain is removed and the corpus allatum retained, ripening occurs normally ; and the blood from a female with a corpus allatum will induce egg development when transfused into a female without. In the male *Rhodnius* the presence of the corpus allatum seems to be necessary for the normal activity of the accessory glands, and the blood of a male with the corpus allatum intact will induce egg formation in a decapitated female. It would appear from these

experiments that the corpus allatum in the adult *Rhodnius* produces a hormone necessary for sexual maturity. This hormone seems to differ from that present in the nymphs during moulting (p. 37); for the blood of growing nymphs does not induce ripening of eggs in the adult.[168] And in the grasshopper *Melanoplus*, if the corpora allata are removed from females soon after moulting, the oöcytes fail to ripen, and the secretion of the anterior segment of the oviducts, which forms the oötheca, is absent.[162]

But this is not a constant function of the corpus allatum. For *Bombyx mori* and other Lepidoptera will develop eggs normally if the corpora allata are excised in the last larval stage [13]; and *Dixippus* (p. 46) from which the corpora allata have been removed in the third or fourth nymphal stage are caused to omit several instars and then produce viable eggs.[119, 120] The females of Coccids,[118] and old termite queens [64] show a great enlargement of the corpora allata; as also do various Lepidoptera during the pupal and imaginal stage [70]; but it is possible that this change is merely a reflection of a high rate of metabolism and does not indicate the secretion of a specific hormone. It is worth noting that some hypertrophy of the corpus allatum occurs also in the soldier caste of termites.[120] The oenocytes in *Rhodnius* show a similar enlargement during egg production which is perhaps to be explained in the same way.[167]

The failure of the ovary in *Blatella* to mature in the nymphal stage has been attributed to impermeability of the oöcytes; and their increased permeability in the adult has been ascribed to hormones secreted by the fat body. The growth of fresh oöcytes is arrested during the carrying of the egg capsule; and this arrest has been attributed to substances in the haemolymph, secreted perhaps by the corpus luteum.[71]

Absorption of oöcytes—In *Rhodnius* females which are starved, or deprived of the corpora allata, the oöcytes begin their development in the usual way. But when they are part grown, and are separated from the nurse cells, they die. The follicular cells then proliferate amitotically in a disorganized manner, invade and absorb the dead oöcyte, and eventually undergo necrosis and absorption themselves like the corpus luteum of the normal ovariole (Figs. 308, 309).[168] The same change is seen in the ovaries of workers among Hymenoptera. In workers of *Formica rufa* and *Camponotus ligniperda* the eggs develop as in the queen up to the stage of yolk formation; they then die and are reabsorbed.[163] The same changes are seen occasionally in the queens; they affect a certain number of the oöcytes in normal mosquitos [107]; and they occur in Pteromalids, either spontaneously (a condition known as 'phasic castration') or if they are prevented from ovipositing.[38] Whether this regression of the ovaries is an effect of nutrition, of metabolism, or of a specific hormone is uncertain. Probably it results from the interaction of all these factors.

Hibernation and diapause—Reproduction, like growth, may suffer a periodic arrest; and as with growth (p. 67) this arrest may be a direct effect of an adverse environment, or it may be a true 'diapause' which persists even under favourable conditions. In *Dytiscus* and various other beetles, the gonads revert to a resting state after the first reproductive period; they show renewed activity about the same date the following year, sometimes in a third year also. This seems to be a deep-seated rhythm and not a simple effect of warmth following the winter cold; for in *Carabus coriaceus* and *Leistus* spp. the breeding season does not begin until the late summer or autumn.[35]

Mosquitos become more or less immobile in the autumn ; and after taking meals of blood they tend to develop an enlarged fat body instead of maturing their ovaries (' gonotrophic dissociation ').[151] In *Culex pipiens*, this change seems to be closely related with the temperature : if given blood and kept warm at any time in the winter they will develop eggs.[58] But if *Anopheles maculipennis* race *atroparvus* is fed with blood during the winter and kept at 29° C., although some develop eggs, others lay down fat body.[58, 151] And *A. maculipennis* race *messeae* in the autumn shows complete ' gonotrophic dissociation ' ; it fails to produce eggs at any temperature.[8] In this state there is no absorption of developing oöcytes as described in the last section ; the ovaries show no signs of growth at all.[169]

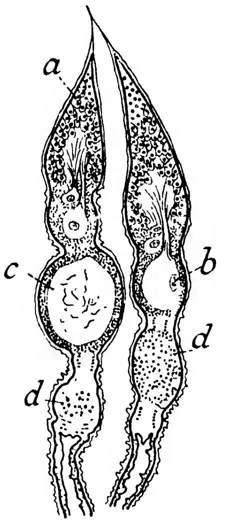

FIG. 308.—Ovarioles of *Rhodnius* deprived of corpus allatum (*cf.* Fig. 296) (*after* WIGGLESWORTH)

a, nurse cells ; *b*, live oöcyte still nourished by nutritive cord from the nurse cells ; *c*, dead oöcyte ; *d*, autolysing residues of dead oöcytes and follicles.

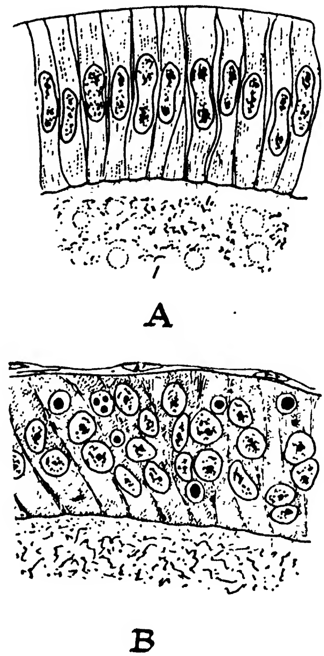

FIG. 309

A, healthy follicular epithelium around normal oöcyte in *Rhodnius* ; *B*, degenerating follicular epithelium around dead oöcyte, in *Rhodnius* deprived of corpus allatum (*after* WIGGLESWORTH).

SPECIAL MODES OF REPRODUCTION.

Polyembryony—Some parasitic Hymenoptera show a peculiar form of asexual multiplication in which a single egg divides in the course of development to produce a number of embryos. This condition, described originally by Marchal,[92] has arisen independently among Chalcididae, Proctotrypidae, Vespoidea, Braconidae and Ichneumonidae.[69, 115] It is seen in its simplest form in *Platygaster hiemalis*, a parasite of the Hessian fly (Fig. 310). After maturation of the egg in this insect, the two polar bodies fuse into a single polar nucleus, which divides amitotically to form a ' paranuclear mass '. This ultimately grows around the germ to produce a nutritive sheath or ' trophamnion ', which obtains nourishment from the tissues of the host and gives it up to the embryo. As in all endoparasitic Hymenoptera the egg is very poor in

yolk, and cleavage is complete. When division has reached the 4-cell stage, the germ and the paranuclear mass may either split into two and give rise to two embryos, or they may fail to divide and so develop into a single larva.[87] By the same process the egg of *P. vernalis* gives rise to an average of eight individuals.[87]

In *Macrocentrus gifuensis*, a Braconid parasite of the European corn borer, the primary embryonic germ divides by fission to form two secondary germs each accompanied by half the paranucleus; these secondary germs split up into a variable number of morulae, each of which develops into an embryo. Some of the morulae remain grouped in 'polygerms' of 2–6; many become isolated. When the larva is fully formed, the trophamnion is very thin and reduced; the larva then straightens out its body and gnaws its way out.[115] In the Chalcid *Encyrtus fuscicollis* a chain of 100 or so embryos arises; here the

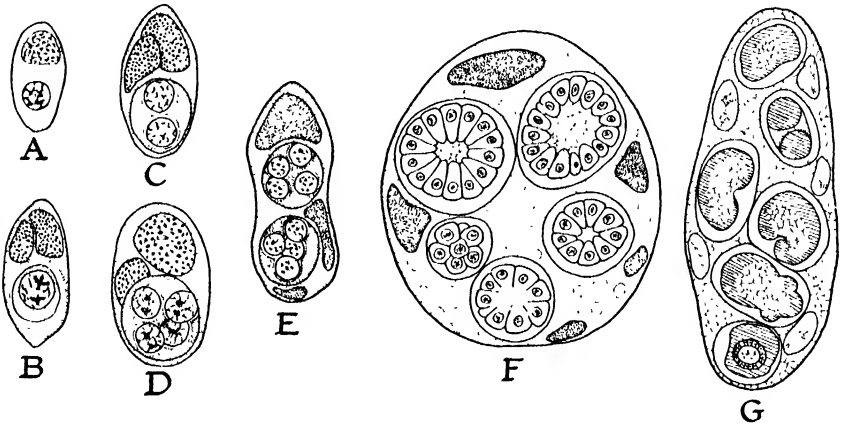

FIG. 310.—Polyembryony in *Platygaster* (*after* LEIBY and HILL)

A–E, *Platygaster hiemalis*; *F*, *G*, *P. vernalis*. *A*, fertilized egg 24 hours old, showing polar nucleus above and segmentation nucleus below. *B*, egg about 2 days old; polar nucleus divided into two paranuclear masses; embryonic region containing segmentation nucleus is now differentiated from the trophamnion. *C*, parasite body about 3 days old; two embryonic nuclei in the embryonic region. *D*, parasite body 4 or 5 days old; four embryonic nuclei present. *E*, embryonic region divided to form twin germs with trophamnion infiltrated between them. *F*, 18-day-old parasite in polyblastula stage; five embryos at early blastula stage; trophamnion vacuolated. *G*, polyembryonal mass at about 26 days, showing portions of six embryos in process of forming their germ layers; trophamnion less dense, paranuclear masses less conspicuous.

blastomeres after breaking up into morulae, each invested by trophamnion, are massed in a single germinal tube.[94] In the Chalcid *Litomastix truncatellus*, developing in the larva of *Plusia gamma*, as many as 2,000 individuals may arise from a single egg.[146] In addition to the trophamnion described above, *Litomastix* has an inner membrane or 'pseudoserosa' formed by delamination of the blastoderm at the morula stage.[146] These membranes usually disintegrate about the time the larva becomes active, but in *Apantales glomeratus* [47] a fenestrated envelope encloses the larva throughout its life. In some cases a cellular sheath is also laid down by the host.[69]

Viviparity—The eggs in some insects may be retained within the genital tract of the mother until development is well advanced. In *Cimex*, as we have seen (p. 390), fertilization takes place in the ovary, and the embryo has almost reached the stage of blastokinesis by the time the egg is laid [1, 25]; and in *Lecanium hesperidum* and other Coccids, the young nymphs may hatch almost

immediately after oviposition.[161] This is sometimes called 'ovoviviparity'. It may be seen occasionally among such oviparous Diptera as *Calliphora*, *Pycnosoma*, *Musca*, *Phora*, &c., if the eggs are held back from any cause.[74] It occurs normally among various Tachinids, in which the uterus may increase enormously in extent during the gestation of the eggs.[74] In *Panzeria rudis*, for example, the eggs collect in regular transverse rows, and the uterus becomes so extensive that it is thrown into three or more coils like a snail shell (Fig. 311). Fertilization takes place at the anterior end of the uterus; all intermediate stages of development can be seen, the eggs at the lower end containing fully formed larvae which escape from the chorion during oviposition.[122] In Sarcophagidae and some Oestridae a smaller number of eggs are present, and they may hatch inside the uterus.[74] In other Diptera, such as *Theria muscaria*, *Musca larvipara*, *Dasyphora pratorum*, *Mesembrina meridiana*, &c., only one

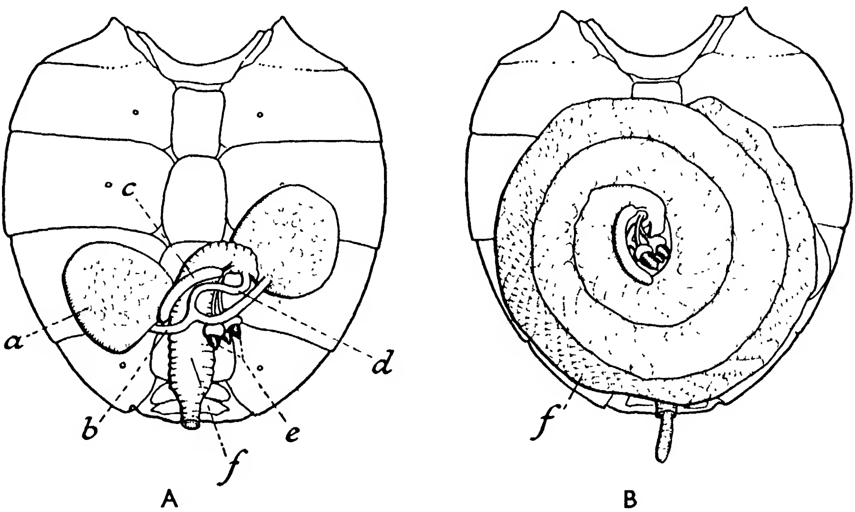

FIG. 311.—Ovoviviparity in the Tachinid *Panzeria rudis* (*after* PRELL)

A, sexual apparatus of newly emerged female; *B*, the same in mature female, showing the vagina hypertrophied to form brood chamber, and eggs containing fully formed larvae being deposited. *a*, ovary; *b*, oviduct; *c*, accessory gland; *d*, common oviduct or uterus; *e*, receptacula seminis; *f*, vagina.

large egg passes at a time into the dilated uterus; in the last named species the egg measures 4·5–5 mm. in length. In this group each egg hatches at the time of laying; in *Hylemyia strigosa* the cuticle of the 2nd larval instar is already formed by this time, and the larva moults to the 2nd stage before feeding.[74] In the aberrant fly *Termitoxenia*, which lives in the nests of termites, the egg is relatively still larger; it hatches immediately after laying, and the larva enters the pupal rest a few minutes later.[99]

In none of these cases does the embryo receive any nourishment from the mother during its stay in the uterus. But in *Glossina* [134] and the Pupipara [55] the larva, which hatches in the uterus from an egg of normal size, is nourished until it is fully grown by special 'milk' glands, probably modified accessory glands, which ramify throughout the abdomen and open on a papilla close to the mouth of the larva (Fig. 312). These larvae are matured singly, breathing by the extrusion of their posterior spiracles through the female opening. They

are already immobile ' prepupae ' when deposited, in the Pupipara ; in *Glossina* they become immobile within a few hours without feeding.

In *Cloëon dipterum* the eggs, which have an exceedingly delicate chorion, are not deposited immediately after mating as in most other Ephemeroptera, but are retained in the oviducts. Here they remain for 10–14 days ; and then the female seeks the water and deposits the newly hatched nymphs.[9]

In other groups the developing eggs may be retained in the ovarioles, as in some Chrysomelids,[63, 127] Coccids, Aphids. In all such cases the chorion is absent. In Coccids and Aphids the nutritive cords continue to supply nourishment during early segmentation ; later, the follicular epithelium takes on this function ; the thin amnion and serosa do not interfere.[161] In the parasitic Orthopteron *Hemimerus*, the egg begins to develop as soon as it enters the basal part of the ovariole. The follicular epithelium swells up at each end into a rounded mass. Within these masses, as elsewhere in the epithelium, the cells dissolve and the nuclei break down, and the growing embryo enclosed in serosa and amnion absorbs the nutrient substances so produced (Fig. 313).[59, 161] This enlargement of the follicular epithelium is sometimes described as a sort of ' placenta '. The same term might equally be applied to the general follicular epithelium of viviparous Aphids and Coccids.

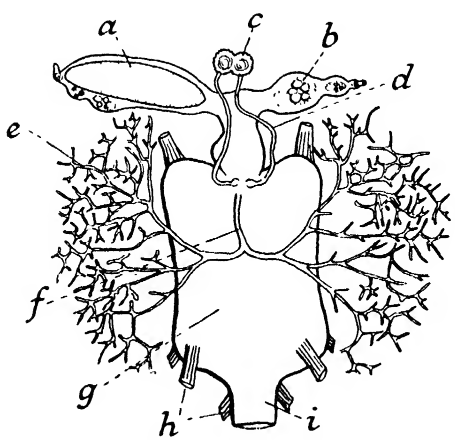

FIG. 312.—Female reproductive system in *Glossina* (*after* ROUBAUD)

a, nearly mature egg in ovary ; b, egg due to mature next ; c, spermathecae containing sperm ; d, duct of spermatheca ; e, accessory or uterine glands ; f, common duct of these glands ; g, uterus ; h, uterine muscles ; i, vagina.

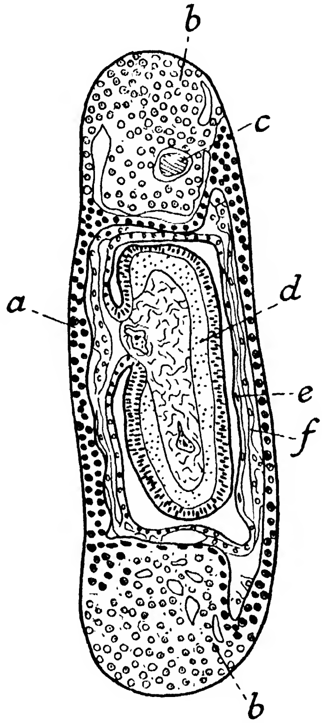

FIG. 313.—Longitudinal section of ovarial follicle in *Hemimerus talpoides* (*after* HEYMONS)

a, follicular epithelium ; b, follicular epithelium hypertrophied to form a nutritive mass or ' placenta ' ; c, nutritive material produced from the breakdown of these cells ; d, embryo ; e, amnion ; f, serosa.

A more definite type of ' pseudo-placenta ' is seen in the viviparous Polyctenid *Hesperoctenes*. Here the ova are yolk-free and devoid of chorion. Cleavage is complete and begins in the ovariole, the embryonic membranes being formed as usual. Until blastokinesis a ' trophoserosa ' surrounds the embryo ; the pleuropodia (p. 11) then begin to grow outwards, and fuse over

the surface so that the embryo is completely invested by a ' pleuropodial membrane '. It is this membrane which functions as a placenta, collecting nourishment from the egg tube as the embryo passes down it, but never attached to the wall.[51]

In a few cases, embryonic development takes place in the general body cavity of the mother, as in the paedogenetic forms to be considered later, and in Strepsiptera. The ripe female of Strepsiptera is little more than a sac with a few vestigial organs and a great quantity of fat body in which the immature embryos, nourished by means of a ' trophamnion ', are imbedded. When these are mature, they escape as triangulin larvae through a series of unpaired ventral genital canals, and make their way out through the ' brood canal ', a cleft between the body of the female and the unshed larval and pupal skins.[60]

Neoteny and paedogenesis—We have already noted several abnormal cases of neoteny, that is, the retention of youthful characters beyond the usual period in ontogeny (p. 47).[175] Among Coccids this condition is normal ; the females, which always have at least one less moult than the males, retain a wingless form approximating to that of the nymphs.[161] And the females of various Malacoderm beetles, for instance Lampyridae, retain the larval form to a varying extent. An extreme example of this is seen in certain Lycid beetles of Borneo, of which the females are the giant ' Trilobite ' larvae, the males are normal and much smaller.[100] Among Protermitidae the royal pair may be either perfect insects which have swarmed and shed their wings, or nymphs, with or without wing rudiments, which have become sexually mature.[48]

This last example approximates to *paedogenesis*, that is, precocious reproduction in the larval or pupal stages. Paedogenesis is usually associated with parthenogenesis (p. 400) ; but an exception is afforded by the viviparous Polyctenid *Hesperoctenes* (p. 398) in which some of the female last stage nymphs may already have spermatozoa in their body cavity, and developing embryos in their ovaries.[51] Most paedogenetic insects are also viviparous : the beetle *Micromalthus debilis* produces larvae with long slender legs which moult into a legless stage ; these legless larvae may either transform into pupae and winged adults, or, by viviparous reproduction, give rise again to legged or caraboid larvae.[6, 123, 140] But an exception is seen in the Chironomid *Tanytarsus boiemicus* ; this is normally parthenogenetic as an adult, but it may begin to lay its eggs while in the pupal stage.[174]

The classic examples of paedogenesis occur among Cecidomyidae and Chironomidae. The phenomenon was first observed by Wagner in 1861 in larvae of *Miastor metroloas*. In these larvae,[41, 147] in larvae of *Oligarces*,[56, 157] and in pupae of the Chironomid *Tanytarsus grimmi*,[158] the oöcytes develop parthenogenetically within the ovarioles until a variable number of daughter larvae are produced ; these are set free into the body cavity, where they rapidly destroy the tissues of their maternal host and ultimately escape through the body wall. As we shall see later, this cycle occurs alongside a normal cycle of metamorphosis and bisexual reproduction (p. 403).

Hermaphroditism occurs in a few insects. In the Plecopteron *Perla marginata* the condition is merely rudimentary, the male nymphs having vestigial ovarioles alongside the testis.[138] But the curious fly *Termitoxenia* is a functional hermaphrodite. It has a female system with two ovarioles, paired

oviducts, uterus and vagina opening on the 8th segment, and a male system consisting of an unpaired testis, vas deferens and penis opening on the 9th segment. Sperm are present only in young individuals; so presumably the male phase precedes the female.[99] And the Californian race of the Coccid *Icerya purchasi* is both hermaphrodite and self-fertilizing. Two classes of individuals exist: 90–99 per cent. of the population are hermaphrodites, the rest are males; no true females occur, though the hermaphrodites retain the instincts and body form of females, and may copulate with the rare males. In the 1st instar of the hermaphrodite nymphs, certain of the more centrally located cells of the gonads, which are still undifferentiated as to sex, undergo reduction from the diploid to the haploid condition (*cf.* p. 401). These haploid cells form the core of the gonad and give rise to sperm; the more peripheral cells remain diploid and give rise to follicles, nurse cells, and oöcytes. All the eggs show normal maturation, with reduction to the haploid chromosome number. Most of them are fertilized by sperm from the same individual and produce diploid hermaphrodites; occasional eggs escape fertilization and develop parthenogenetically to haploid males. Hermaphroditism is here incomplete, in the sense that the form with both types of gonad can never function as a male and fertilize other individuals.[67] Intersexes and gynandromorphs, which are discussed elsewhere (p. 56), although their gonads may be of mixed sex, seem never to be functional hermaphrodites.

Parthenogenesis—We have already noted several examples of development in unfertilized eggs. This may happen occasionally in a species normally reproducing bisexually (*sporadic parthenogenesis*); or it may constitute the normal mode of reproduction (*constant parthenogenesis*); and all intermediate degrees may exist between these two extremes. Sometimes one or more parthenogenetic or agamic generations may alternate with bisexual, gamic or amphigonic generations (*cyclical parthenogenesis* or *heterogony*). The unfertilized eggs may give rise solely to females (*thelytokous parthenogenesis*) or solely to males (*arrhenotokous parthenogenesis*) or to either sex (*amphitokous parthenogenesis*); and the individuals so developed may contain half the normal number of chromosomes in their somatic cells (*haploid parthenogenesis*) or the normal diploid number (*diploid parthenogenesis*).

Sporadic parthenogenesis—In many species of Acridiidae, development begins in nearly all unfertilized eggs; but it is successfully terminated in very few. The haploid female pronucleus divides mitotically giving haploid cleavage nuclei; but in the more successful cases, chromosome division without cytoplasmic division may take place, so that the normal diploid chromosome number is restored. But even when development is complete, many embryos succumb without hatching, or die off as nymphs or young adults. In these eggs, oviposition seems to act as a stimulus for the completion of the maturation divisions; the presence or absence of the sperm nucleus is without effect.[77] A similar condition occurs in Lepidoptera. For example, in the bisexual races of the Psychids *Solenobia triquetrella* and *S. pineti* the diploid chromosome number is restored by nuclear fusion, but hatching never takes place.[142] In many Lepidoptera, however, complete development of unfertilized eggs has been recorded.[24] In *Lymantria*,[46] for example, larvae and adults of both sexes and normal diploid constitution have occasionally been reared in the absence of males; and some of the females of *Orgyia antiqua* lay viable eggs in nature

without mating.[158] As was shown many years ago by Tichomiroff, parthenogenesis may be induced artificially in silkworm eggs by various stimuli ; high temperature, acids, alcohol, various histological fixatives and so forth will serve to activate the eggs.[81]

Parthenogenesis in Phasmids—Among Phasmids, parthenogenesis is universal.[114] In some species it is constant, and males are excessively rare ; other species are ordinarily bisexual, and parthenogenesis occurs only in the absence of the male. In a species like *Dixippus morosus*, which is constantly parthenogenetic, when males appear they are generally intersexes, and rarely pure males capable of successful copulation.[22, 156] These parthenogenetic Phasmids are of three cytological types. (i) In the first parthenogenetic generation of *Menexenus*, many of the cells are haploid, though some are diploid. (In subsequent generations regulation occurs, all the cells are diploid, mortality is less and development more rapid.) (ii) Most species are always diploid. (iii) In a few species, haploid, diploid and polyploid cells may occur together.[22]

Sexual races—Geographical or local races of insects may be characterized by their mode of reproduction. The Psychids *Solenobia triquetrella* and *S. pineti* are represented in different parts of Germany by a normal bisexual race in which the unfertilized eggs never develop completely, and a purely thelytokous parthenogenetic race in which males never appear.[142] In the Coccids *Lecanium hesperidum* and *L. hemisphaericum* there are parthenogenetic races producing only females, and bisexual races with a minority of males. The bisexual races show facultative thelytoky: unfertilized eggs develop into females, fertilized eggs into either sex. In the unfertilized eggs of this race the diploid chromosome number is restored at the outset of development by fusion between the second polar body and the egg nucleus, a process very like normal fertilization. In the parthenogenetic races, the reduction division is omitted, so that the oöcytes are always diploid.[152] In *Trialeurodes vaporarium* there is a race showing arrhenotokous parthenogenesis ; fertilized eggs producing females, unfertilized producing males ; and a race consisting almost exclusively of females (14♂ : 4582♀). In the latter race the diploid constitution is restored after the second maturation division by autoregulation—probably through splitting of chromosomes without nuclear division.[152]

Haploid arrhenotokous parthenogenesis—It is evident from the foregoing examples that most insects will develop normally only when the diploid constitution has been regained, but haploid parthenogenesis is a constant occurrence in a few cases. The best known example is the honey-bee and other Hymenoptera, in which the eggs undergo normal maturation, fertilized eggs develop into diploid females, unfertilized into haploid males.[158] The same condition exactly is seen in *Aleurodes proletella*,[152] in one of the local races of *Trialeurodes vaporarium*[152] and in *Icerya purchasi*, *I. littoralis* and related forms—in which the somatic cells of the female contain 4 chromosomes, the males 2.[67]

Cyclical parthenogenesis in Cynipids—Alternation of bisexual and parthenogenetic generations occurs in its most regular form in Cynipids. The two generations often attack different parts of the host plant, and may differ so strikingly that in many cases they have been regarded as separate species. In the oak-gall wasp *Neuroterus lenticularis*, all the fertilized eggs, derived from the bisexual generation, develop in the early spring into females which

reproduce parthenogenetically in the early summer, some producing only sexual males, others only sexual females. In the female-producing eggs the maturation divisions are omitted, consequently they have the diploid number of chromosomes; in the male-producing eggs reduction occurs as usual, the males are therefore haploid as in other Hymenoptera. Fertilization of the eggs of this generation furnishes the females of the following spring.[31]

Cyclical parthenogenesis among Aphids—The life cycles of Aphids are complicated by an increase in the number of successive agamic generations, frequently by a periodic change of host plant, by polymorphic wing development, and by the association of parthenogenesis with viviparity. In a simple form such as *Aphis rumicis*, the winter egg gives rise to an agamic, usually wingless, female, the fundatrix, which produces viviparously winged and wingless agamic females (fundatrigenia). The winged forms spread to other specimens of the host plant and again, like their wingless sisters, produce viviparously winged and wingless agamic females. These parthenogenetic viviparous generations continue until the autumn, when, among the agamic females, there appear some (sexuparae) which give rise to winged and wingless males and amphigonic oviparous females (sexuales) which lay a small number of fertilized winter eggs.[159] But the cycle may be far more complicated where the insect changes from one type of host plant to another.[101]

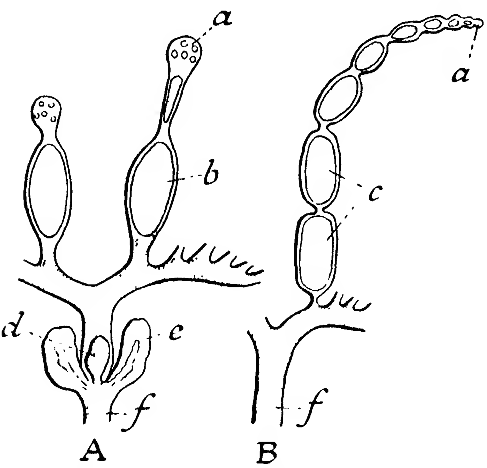

FIG. 314.—Female reproductive system in the Aphid *Macrosiphum solanifolii*

A, gamic type; *B*, parthenogenetic type. (*After* SHULL.) *a*, germarium; *b*, egg; *c*, embryos; *d*, seminal receptacle; *e*, colleterial gland; *f*, vagina.

In *Pemphigus*, *Phylloxera*, *Chermes*, viviparity does not occur, the amphigonic generation is always wingless and dwarf, and the amphigonic female produces only one egg. *Phylloxera vastatrix* may be noted as an example. The winter egg, laid on the branches of the grape vine, hatches to produce a fundatrix female, forming a gall on the leaf, which gives rise parthenogenetically to a series of gall forming generations ('gallicolae'). In the course of the summer, an increasing proportion of individuals with a longer proboscis are produced, and these migrate to the roots where they continue to reproduce parthenogenetically ('radicolae'). Towards autumn they give rise to individuals which undergo four moults (instead of three as in the 'gallicolae' and 'radicolae') and develop into winged adults. These are 'sexuparae'; they lay eggs which give rise to sexual males or sexual females, a given sexupara usually producing only one sex. The sexual forms are apterous, the proboscis is absent and the gut rudimentary. After mating, the female lays a single large winter egg.[158]

The reproductive system may differ markedly in the different phases of these life cycles. For example, in *Macrosiphum solanifolii* the reproductive system of the amphigonic females consists of a vagina with a pair of colleterial

glands and seminal receptacle, a pair of short oviducts and usually 10 ovarioles. There are never more than two oöcytes in each ovariole, and these are surmounted by a large spherical germarium (Fig. 314, A.) In the parthenogenetic females, the accessory glands and seminal receptacle are absent; the ovarioles, which vary in number, each contain 6–9 embryos, eggs, or oöcytes, and bear at the end a very small germarium (Fig. 314, B).[144]

We have discussed in an earlier chapter the mechanisms by which the body form and type of reproduction in Aphids is determined by environmental factors (p. 61). In some Aphids living in the uniform environment of the tropics or of hot-houses, the amphigonic generations have disappeared from the life cycle and they show continuous parthenogenesis.

Alternation of generations in Cecidomyids—The cycle of development in *Miastor* and *Oligarces* (Fig. 315) shows a degree of instability which is unparalleled elsewhere among insects. Under the conditions of abundant food, high humidity and constant temperature which obtain in the dark depths of the rotten tree stumps in which *Miastor* larvae occur, paedogenetic reproduction will continue indefinitely.[41, 147] Exposure to light [147] or desiccation [41] may cause these larvae to enter a kind of diapause; they become orange in colour, develop various structural distinguishing characters, acquire a migratory habit, and fail to grow or reproduce. If the ordinary white larvae are starved, these orange larvae appear in the next generation.[41] If the orange larvae are given abundant food, they are 'rejuvenated' and their sexual products mature. If they are ligatured or injured by burning, activation and maturation of the oöcytes is similarly induced.[41]

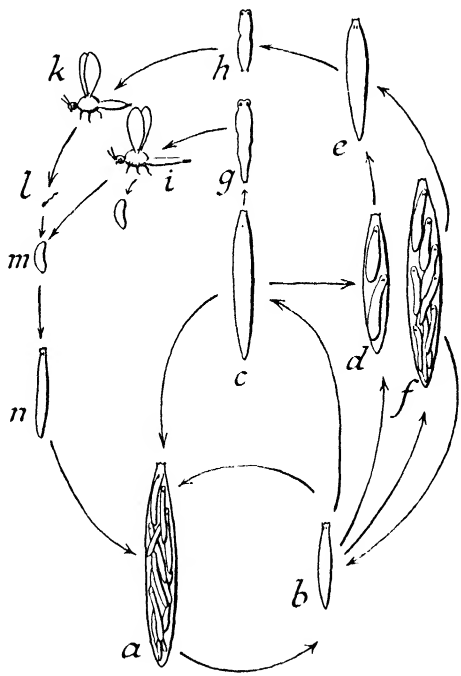

FIG. 315.—Alternative reproductive cycles in *Oligarces paradoxus* (*after* ULRICH)

a, paedogenetic larva giving rise to *b*, undetermined daughter larva which may develop in *a* again or into *c*, female imago-producing larva, or into *d*, which gives rise to male imago-producing larvae (*e*) or into *f*, which gives rise both to undetermined daughter larvae and to male imago-producing larvae; *g*, female pupa; *h*, male pupa; *i*, female imago; *k*, male imago; *l*, sperm; *m*, egg; *n*, young larva from egg.

If white larvae of *Miastor*, growing very slowly at 5–10° C., are suddenly exposed to 30° C., they often give rise to an egg which produces a larva which transforms to a pupa and adult.[41] Sometimes larvae of an intermediate size may produce by paedogenesis 2–5 of these pupating larvae.[147] In *Oligarces*, pupating larvae can be induced at will by overcrowding the previous generation [56]; or, if they are fed on a culture of moulds, more pupating larvae are induced, either directly or in the offspring, as the culture gets older; thus a culture of moulds 2 days old gave 0·5 per cent. of pupae, a 7-day culture 14·4 per

cent., a 12-day culture 41·8 per cent.[157] The newly-born pupa-forming larvae have imaginal discs (which are always absent from paedogenetic larvae) already visible; yet by transferring them to a fresh culture as soon as they are detected, their type of development may be reversed, the discs vanish and they grow into paedogenetic larvae [56]; in *Miastor*, this reversal may be induced shortly before metamorphosis by burning the head or genital region.[41]

Since the paedogenetic larvae are all females, while the pupating larvae may be of either sex, it follows that the sex of these larvae is highly indeterminate also. In the parthenogenetic eggs of the paedogenetic larvae, the reduction division is omitted at maturation, so that the egg nucleus has the diploid chromosome number from the outset.[73]

SEX DETERMINATION

Sex is determined by the distribution of the heterochromosomes or sex chromosomes. These may be of several types. (i) There may be an unpaired *X*-chromosome or monosome which occurs singly in the diploid state in one sex. In Orthoptera, Homoptera, some Heteroptera, a few Diptera and some Coleoptera and Neuroptera, the monosome occurs in the male. The female then has two *X*-chromosomes. The monosome occurs in the female of many Lepidoptera, e.g. *Talaeporia*,[141] *Ephestia*.[83] (ii) The *X*-chromosome in one sex may be paired with a chromosome which often differs morphologically from itself. A dissimilar pair of this kind, called *X* and *Y*, and termed idiochromosomes or diplosomes, occurs in the males of most Heteroptera, Diptera and Coleoptera, in the females of which two *X*-chromosomes are present. In many Lepidoptera, e.g. *Lymantria*, the females have the *XY* constitution.

The mode of action of these chromosomes in controlling sex during development has been considered in an earlier chapter (p. 56). Here we are concerned with those disturbances in the sex ratio which result from unusual distribution of the sex chromosomes at maturation and fertilization.

Normally, equal numbers of *X* and *O* or *X* and *Y* gametes will be produced at meiosis in the heterogametic sex; consequently, equal numbers of *XX* and *XO* or *XX* and *XY* zygotes will result at fertilization, and the sex ratio will be equal. But in females of the Psychid *Talaeporia*, which have the *XO* constitution, high temperature and over-ripeness of the eggs cause the *X*-chromosome to migrate more frequently into the female pronucleus. Hence more *XX* individuals, i.e. males, arise at fertilization. At low temperature the *X*-chromosome goes more often to the polar body, and therefore more females are produced. Thus the sex ratio ranges from 100♀ : 162♂ at 30–37° C. to 100♀ : 65♂ at 3–8·5° C.[141] In *Ephestia*, delayed fertilization has no effect on the sex ratio.[72] In *Drosophila*, an excess of females appears among the latest eggs to be fertilized.[85] In the Coccid *Pseudococcus citri*, delayed fertilization results in a great preponderance of males: the ratio may range from the normal 101♂ : 100♀ if fertilization is immediate, to 991♂ : 100♀ after 10 weeks delay.[72] In this case, since the male is the heterogametic sex, the cytological explanation must be different from that in *Talaeporia*. Families consisting almost entirely of females in *Abraxas grossulariata* are due to a gene which causes passage of the *X*-chromosome to the second polar body during the maturation of nearly

all the ova [24]; in *Lymantria dispar* such families are due to a dominant lethal gene which kills nearly all the males.[24]

In the amphigonic generations of Aphids, as we have seen, all the offspring are female. That is because during the maturation of the sperm the male-producing spermatids degenerate; all the functional spermatozoa therefore carry the X-chromosome and are female-producing.[5, 86, 102]

In the parthenogenetic generations of Aphids, the females of which have the XX constitution, only one division occurs at maturation, and there is no reduction. The egg therefore retains the XX constitution of the mother, and consequently the offspring are generally female. But occasionally one of the X-chromosomes fails to divide (non-disjunction) and passes to the polar body. Consequently the egg acquires the XO constitution of the male. In many species a given female of the sexuparous generation is either solely male-producing or solely female-producing [102, 143]; in *Pemphigus spirothecae* a single sexupara produces always males from the two anterior egg tubes, and females from the more caudal egg tubes.[155]

In most insects, since the female is homogametic (XX), it is easy to see that in parthenogenetic forms, in whatever way the diploid constitution is restored, the offspring will also be XX or female (thelytokous parthenogenesis). Whereas in the sporadic parthenogenesis of Lepidoptera, with females XO or XY, either the X or the Y may predominate in the offspring and, as we have seen, both sexes are produced. But in those races of *Solenobia* (p. 401) which show *constant* parthenogenesis this is purely thelytokous. That is because in these races the X-chromosome always passes into the polar body at the first maturation division; consequently the Y always predominates and the offspring are always female.[142]

In the honey-bee, and many other Hymenoptera, all the fertilized eggs become females, the unfertilized become males (Dzierzon's law). All the sperm are in fact female-producing, though the cytological explanation of this must be different from that in the Aphids described above. Thus the control of sex in Hymenoptera resolves itself into control of fertilization.* The 'voluntary' control of the muscular wall of the oviduct orientating the egg towards the receptacular duct, and the control of the so-called sperm pump or sphincter of the duct, regulate the entry of sperm into the egg.[2] In the honey-bee it is uncertain what stimulus determines this response; possibly it is the size of the brood cell; but to some extent the regulation is seasonal, male eggs being produced in the autumn.[158] The female *Osmia* constructs large cells, and deposits fertilized eggs in them, as soon as the seminal receptacle is filled with sperm; whereas the virgin female, or the female whose supply of sperm is exhausted, constructs small cells and lays unfertilized male eggs.[28] The parasitic *Typhia*, which oviposits in 2nd and 3rd instar larvae of *Popillia* has a normal sex ratio with a slight preponderance of females when it develops in 3rd instar larvae; there is a preponderance of males when it develops in 2nd instar larvae; and this difference persists when the eggs after laying are transferred to 3rd instar larvae. Here the sex is clearly determined by the ovipositing female, perhaps in accordance with the size of host [16] (*cf.* p. 59). A similar

* There are exceptions to this rule. For example, in the Cape variety of *Apis mellifica*, fertile workers are common; and they produce males, workers, and even queens, apparently parthenogenetically.[158]

control exists in the American race of *Trialeurodes vaporarium*, which shows arrhenotokous parthenogenesis: the fertilized eggs becoming female, the unfertilized male; but the mechanism of this control is not known.[139, 152]

TRANSMISSION OF SYMBIOTIC MICRO-ORGANISMS

As we saw in an earlier chapter (p. 288), many insects constantly harbour intracellular micro-organisms. The mutual adaptations between these organisms and their host suggests that the relation between them is symbiotic; and one aspect of reproduction in such insects concerns the diverse mechanisms by which these symbionts are transmitted to the offspring.

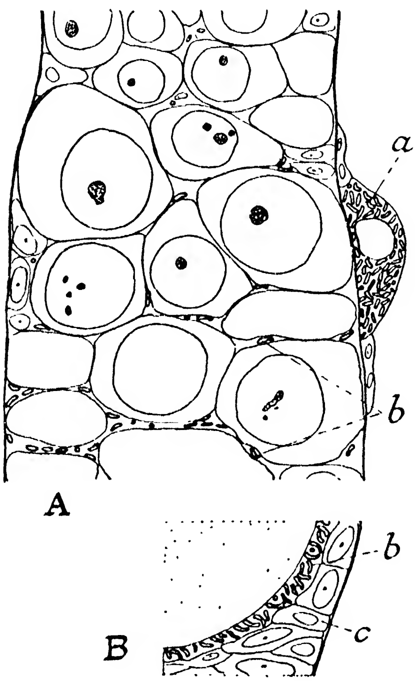

FIG. 316

A, upper end of ovariole of *Periplaneta americana*, showing bacteroids in amycetocyte and between the oöcytes; B, portion of follicle and oöcyte at later stage, showing bacteroids between oöcyte and follicular cells. (*After* GIER.) a, mycetocyte; b, bacteria; c, follicular cells.

In the bed-bug *Cimex*,[17] and the weevil *Apion*,[108] the symbionts invade the nurse cells and are conveyed from them to the oöcytes. In the ant *Camponotus* there is a general infection of the young follicle, leading to a generalized infection of the egg plasma,[17] and in Blattidae the bacterial layer forms similarly over the egg surface, later invading the vitellophags and subsequently the fat body and ovary (Fig. 316).[44] In the egg the infected cells are always the extra-embryonic blastoderm or the yolk cells. In *Oryzaephilus* (Col.),[79] *Lyctus* (Col.),[79] Anoplura (Fig. 294, F., G),[130] the posterior pole of the egg is invaded shortly before secretion of the chorion. In parthenogenetic Aphids the embryo is entered while it is in the ovary.[154] In *Rhizopertha* (Col.) the micro-organisms invade the testis, mix with the sperm, and after being passed with them into the bursa copulatrix of the female, enter the egg through the micropyle during fertilization.[91] The same thing probably happens in the bug *Nezara*, in which bacterial sacs are associated with the male organs.[90] In Trypetidae the organisms pass from the hind gut into the ovipositor, and enter the micropyle of the egg when it is laid [148]; they also enter the micropyle in Lagriidae (Col.).[148] In Anobiid beetles, the yeasts collect in diverticula in the female tract; from here they contaminate the surface of the egg, and the newly hatched larva is infected by eating the shell.[14] The organisms in the modified Malpighian tubes of some Chrysomelid beetles, are deposited with the mucilaginous secretion in the egg cap, which is eaten by the larva,[148] and a similar transmission of the organisms from the gut occurs in the beetles *Bromius* and *Cassida*.[148] Finally, in the Pupipara [173] and *Glossina*,[166] in which the larva is nourished in the uterus until fully grown, the symbionts are transmitted through the 'milk' glands.

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